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[1] J. B. Austin and R. H. H. Pierce, *J. Amer. Chem. Soc.* **55**, 661 (1933).

[2] J. R. Goldsmith, D. L. Graft and O. I. Joensuu, *Geochim. et Cosmochim. Acta* **7**, 221 (1955).

[3] S. T. Yoffe and A. N. Nesmeyanov, *Handbook of Magnesium-Organic Compounds* Vol. II, p. 239. Pergamon Press, London (1956).

Abbreviations are used freely in *Tetrahedron*, particularly in setting the experimental section. For the most part these are not followed by a full stop (period). Among the more commonly used abbreviations, these may be listed:

g kg mm cm ml cc cm^3 sec min hr n_D^{25} $[\alpha]_D^{25}$ 25° (without C which is always understood) but m.p., b.p., l. (litre), in. (inch) but in^3.

New compounds should be indicated by underlining the name (for italics) at its first mention (including headings) in the experimental section only, and by giving analytical results in the form: (Found: C, 62·9; H, 5·4. $C_{18}H_{18}O_6N$ requires: C, 63·2; H 5·3%).

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Proceedings of a Conference on

ROTATORY DISPERSION: RELATED THEORY AND APPLICATION

Edited by

B. H. LEVEDAHL and T. W. JAMES

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20–22 January 1960

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FOREWORD

LIGHT is perhaps the best plaything that the gods could have provided man in his quest for reason and purpose in the universe. This toy at once puts him in communion with atoms and galaxies. It is the object of his artistry and wizardry; his symbol of purity, warmth, and reason. The understanding of it parallels the phylogeny of his mind. He has been seduced by its color, and it has made him the victim of pomp and humility.

In the twentieth century he has responded explosively in applying the discoveries of the previous centuries. But his mastery of application has sometimes dulled his curiosity and what little remains is often too small to substitute for his lack of courage to venture into endeavors which do not promise instant popularity. Too soon he sets his toy in the corner thinking that with it there are no games left to be played. His flighty span of attention leads him to other things because he is still unaware of the catalytic power of well-focused thought.

Perhaps optical rotation and rotatory dispersion is one such game that has remained too long in the corner. Professor Elsasser brought this rude fact to our attention by pointing out that Louis Pasteur presented his now famous work on the rotation of polarized light by tartaric acid crystals one hundred years ago to the day our conference was held (without corrections for intervening leap years). But what has happened in this time? What new rules have come to the game? To the workers who have maintained interest in this subject many things have happened. The turn of the century represents the high point in the development of fundamental concepts for it was then that the observations of Cotton and the analysis of rotatory dispersion by Drude emphasized the beauty of these optical phenomena. From Biot's discovery of the phenomenon in 1815 it again required a hundred years for Born's theory to appear. Kuhn's theory and mathematical treatment was the forerunner to the sophisticated application of quantum mechanics to the subject.

Quantum mechanics is the ultimate in gamesmanship, and in it intuitive jumps are often forbidden transitions. The route from a deterministic to a probabilistic world is a difficult transition to make because the former is included in the latter. This volume will illustrate this difficulty. This was, in fact, the aim of the conference. Rotatory dispersion will be one of the victories of quantum mechanics, and the works of Condon, Eyring, Kauzmann, Kirkwood, and Moffit have sparked the modern attack. The order of events may be the experiment and then the idea, or the idea and then the experiment; either way will be of little consequence to the meaning and understanding of Optical Rotation and Rotatory Dispersion. At least, as this volume will show, experiments are again being done and our only hope is that the gods will again bless us with Kirkwoods and Moffits to put meaning to them.

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ROTATORY DISPERSION AND THE VIBRATING MOMENTUM OF OPTICALLY ACTIVE ABSORPTION BANDS

W. KUHN
Basle, Switzerland

VOL. 13 1961

OPTICAL rotatory power is a phenomenon which has from the time when it was discovered excited the curiosity of many scientists. Optical rotation itself is at first sight a problem of theoretical physics; but its dependence on chemical structure makes it an important object of research in both inorganic and organic chemistry and the occurrence of pure optical antipodes in living organisms gives it a fundamental importance in biochemistry and biology[1] and the question of the origin of optically active substances in nature even touches the problem of the origin of life. The occurrence of the "wrong" antipode in antibiotics and also in small quantities in natural proteins, together with their necessary formation for kinetic and thermodynamic reasons, in the organisms makes it probable that the decrease of the optically active purity of some fundamental substances in the organism will be one of the main reasons of aging and limitation of the lifetime of the individual organisms.

It is not possible in a limited time to follow all the aspects in detail; but it is possible to mention them and to state that they form a unity in spite of the specialization which is characteristic for great parts of todays scientific research.

As the subject of the symposium is optical rotation and rotatory dispersion, we will, in this introductory note, deal with these phenomena; we will see that there is in fact only *one phenomenon*, rotation and rotatory dispersion being intrinsically the same.

I wish, before entering into the main subject to thank the organizing committee, in particular Dr. Levedahl for the invitation to come here and for the great honour which has come to me by being asked to open the symposium. I appreciate the fact that the symposium is dedicated to the memory of Doctors Kirkwood and Moffit who have both made such outstanding contributions in the field which is covered by this conference. The last congress in which optical activity was being the main subject was, as far as I remember, a symposium held by the Faraday[2] Society in 1930. The field has developed in many respects since that date.[3] I believe that the most important progress which was made is the establishment of absolute configuration of the asymmetric carbon atom on one hand (Kuhn, Bijvoet) and the development of the one electron theory (Condon, Eyring, Kauzmann) on the other hand. I also mention the recent investigations on stereoids and experimental development (Djerassi, Klyne).

[1] For a recent review see e.g. W. Kuhn, *Advanc. Enzymol.* **20**, 1 (1958).
[2] *Trans. Faraday Soc.* **26**, 266–461 (1930); with contributions by: P. C. Austin, Belaiew, M. Betti, B. Bhaduri, E. Bretscher, G. Bruhat, A. Cotton, E. Darmois, R. Descamps, C. Drucker, P. P. Ewald, J. Kenyon, W. Kuhn, J. Liquier-Milward, T. M. Lowry, R. Lucas, R. de Mallemann, W. H. Mills, Gl. Owen. H. Phillips, J. Read, H. G. Rule, B. K. Singh, C. P. Snow, G. Temple and K. L. Wolf.
[3] For a recent review see *Ann. Rev. Phys. Chem.* **9**, 417 (1958).

As far as rotatory dispersion is concerned, this property is connected and will always be connected with the fundamental discovery made by Cotton[4] in 1896. He found that optical rotatory power as a function of the wavelength often shows, in the region where the substances show ordinary optical absorption, a characteristic *anomaly* which is associated with a *circular dichroism* in the absorption region and which after the name of its discoverer is called a *Cotton-effect*.

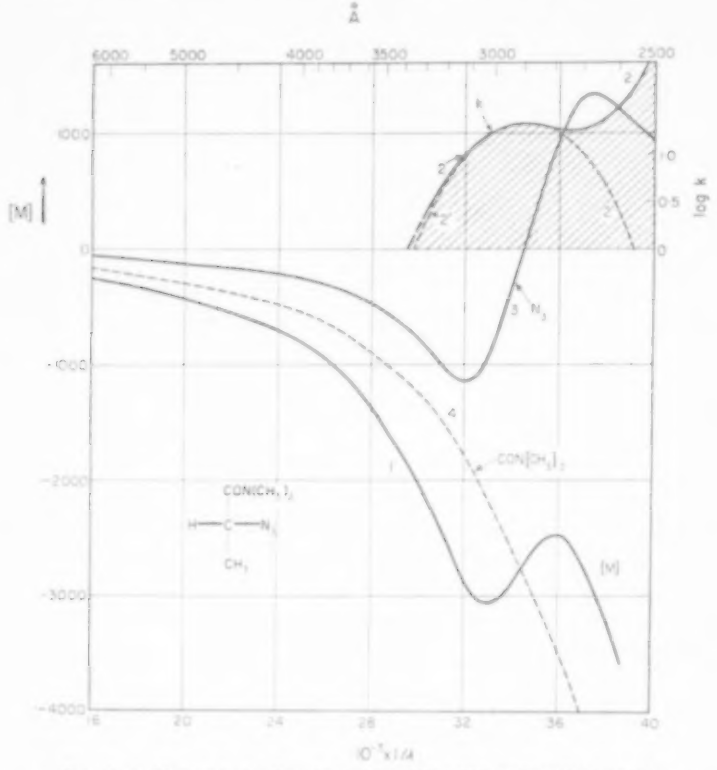


FIG. 1. Azidopropionic-dimethylamide [$CH_3CHN_2CON(CH_3)_2$] in ether.
Curve 1: Molecular rotatory power (M) observed.
Curve 2: Molecular absorption coefficient observed.
Curve 2′: Absorption band (with Gaussian error curve shape), which is used as basis for computing curve 3.
Curve 3: Rotatory contribution of the absorption band (curve 2 or 2′) due to the azido-group.
Curve 4: Difference of curve 1 and 3; significance: rotatory contribution from the remaining absorption bands of the molecule; i.e. essentially the rotatory contribution of the $CON(CH_3)_2$-group.

The first figure[5] shows the situation with respect to rotatory power and optical absorption in the case of D-α-azido propionic dimethylamide. It is the antipode whose Emil Fischer projection formula is indicated in the same figure and whose actual shape, i.e. the absolute configuration[6] is obtained by moving the substituents H and N_3 from the plane of the paper towards the observer. Curve 1 of Fig. 1 is the observed

[4] A. Cotton, *Ann. Chim. Phys.* **8**, 347 (1896).
[5] Taken from W. Kuhn and E. Braun, *Z. Phys. Chem.* (B) **8**, 281 (1930).
[6] W. Kuhn, *Z. Phys. Chem.* (B) **31**, 23 (1935); J. M. Bijvoet, A. F. Peerdeman and A. J. van Bommel, *Nature, Lond.* **168**, 271 (1951); J. Trommel and J. M. Bijvoet, *Acta Cryst.* **7**, 703 (1954).

molecular rotation which shows the Cotton-effect. Curve 2 is the ordinary absorption, a very weak absorption in fact; the f-value or number of dispersion electrons corresponding to about $5 \cdot 10^{-4}$. Fig. 2 gives the ordinary absorption and the circular dichroism[7] of the same molecule in the region of the N_3-absorption band near 2900 Å. It is seen in this figure that the maximum of the circular dichroism practically

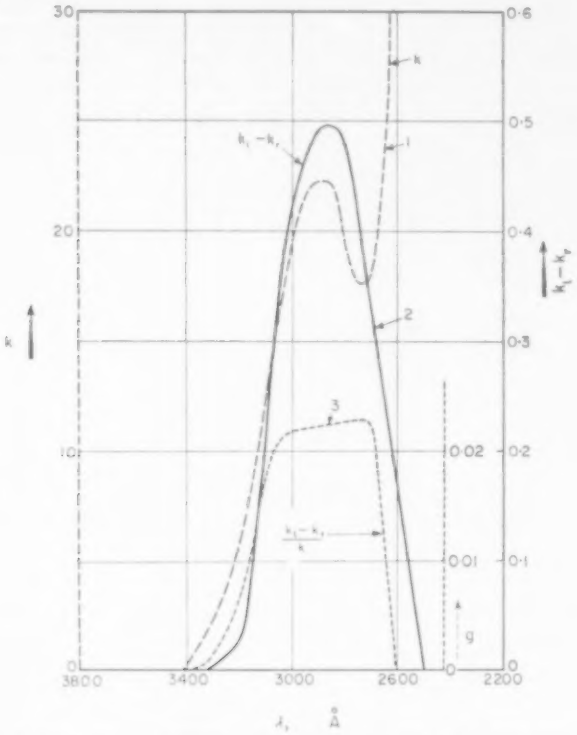


FIG. 2. Azidopropionic-dimethylamide [$CH_3CHN_3CON(CH_3)_2$] in hexane.
Curve 1: Ordinary absorption (k)
Curve 2: Circular dichroism ($k_1 - k_r$)
Curve 3: The anisotropy factor $g = (k_1 - k_r)/k$.

coincides with the maximum of the ordinary absorption and that the anisotropy factor g, i.e. the relative difference of the absorption coefficients for right and left hand circular light, remains roughly constant in the main region of the absorption band.

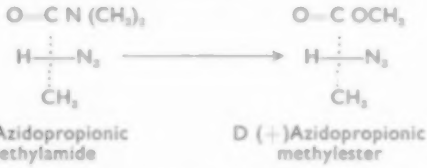
The further analysis has shown[5] that the rotatory anomaly observed both inside and outside the region of absorption is quantitatively connected with the circular dichroism and thereby with the existence of the absorption band. It can in this way be stated in the case of the azidopropionic dimethylamide that curve 3 of Fig. 1 is the contribution of the N_3-absorption band near 2900 Å and that in the visible, e.g. near 6000 Å about 25 per cent of the observed value of the rotatory power are contributed by said absorption band. The *generalization of this statement* is that each absorption band gives a well defined contribution to the molecular rotatory power

[7] W. Kuhn and E. Braun, *Z. Phys. Chem.* (B) **8**, 445 (1930).

not only inside but also outside the absorption region and moreover that the rotatory power of the substance at a given wavelength is the algebraic sum of the rotatory contributions made by the various absorption bands of the molecule.

This is an important statement telling us *that any understanding of the dependence of optical rotatory power on chemical constitution must necessarily proceed through a description or understanding of the Cotton-effect*, i.e. rotatory contributions of the absorption bands exhibited by the substances considered. If we want to understand the influence on optical rotation of a chemical change made in a given molecule we must necessarily ask for the effect produced by the chemical change on the Cotton-effect of the absorption bands. We will in the following consider some of the results obtained on this basis. The azido-compounds have played a particular part in these investigations which we have carried out some years ago in following a proposition of and in close collaboration with Freudenberg[8]. As far as the theory is concerned, I mention the fundamental work which has been done by Oseen[9], Born[10] and later by Condon, Eyring[11] and Kirkwood[12].

It is seen, that we can in a simple way and without changing the arrangement of the substituents attached to the asymmetric carbon atom pass from the azidopropionic dimethylamide to the azidopropionic methylester



D (−)Azidopropionic dimethylamide — D (+)Azidopropionic methylester

Both compounds contain in particular the N_3-group which has an absorption band near 2900 Å and we can, by measuring the rotatory dispersion in the absorption region, or also by measuring the circular dichroism in the absorption band, determine the rotatory contribution of the N_3 absorption band in the ester. The result is shown in Fig. 3. Having made this analysis as well in the dimethylamide (Fig. 1) as in the ester (Fig. 3) we are thus in a position to compare besides the total rotation the effect of the chemical change on the rotatory contribution of a specific absorption band of a specific group (the N_3-group) which remained unaltered by the chemical change. The comparison shows that the rotatory contribution of the N_3-group is negative on the long wavelength side of the N_3-absorption band; we call this a negative Cotton-effect of the N_3-band, both in the ester and in the corresponding dimethylamide. There is a change in the absolute magnitude but, roughly speaking, *a conservation of the rotatory contribution of the N_3-absorption band when going from the ester to the corresponding dimethylamide.*

In contrast to the moderate change produced in the optically active behaviour

[8] For a summary see: W. Kuhn und K. Freudenberg in *Hand und Jahrbuch der Chemischen Physik* Bd. 8/III (1932) and K. Freudenberg, *Stereochemie* pp. 662–720. Wien (1933).
[9] C. W. Oseen, *Ann. Phys.* **48**, 1 (1915).
[10] M. Born, *Phys. Z.* **16**, 251 (1915); *Ann. Phys.* **55**, 177 (1918).
[11] E. U. Condon, W. Altar and H. Eyring, *J. Chem. Phys.* **5**, 753 (1937); E. U. Condon, *Rev. Mod. Phys.* **9**, 432 (1937); E. Gorin, J. Walter and H. Eyring, *J. Chem. Phys.* **6**, 824 (1938); W. J. Kauzmann, J. E. Walter and H. Eyring, *Chem. Rev.* **26**, 339 (1940).
[12] J. Kirkwood, *J. Chem. Phys.* **5**, 479 (1937); **7**, 139 (1939); E. Hückel, *Z. Elektrochem.* **50**, 13, (1944). [The arguments developed in these papers (polarizability theory of optical rotatory power) are formally different but physically about the same as those described by W. Kuhn.]

of the N_3-absorption band, the effect of the chemical change on the total rotation of the molecule is enormous. For the mercury yellow-line 5780 Å a change of the molecular rotation from $+24°$ in the ester to $-259°$ in the dimethylamide is registered. This enormous change is, as we see *not* due to a change of the N_3-contribution; the analysis of the dispersion curves proves that the change is due to the optically active

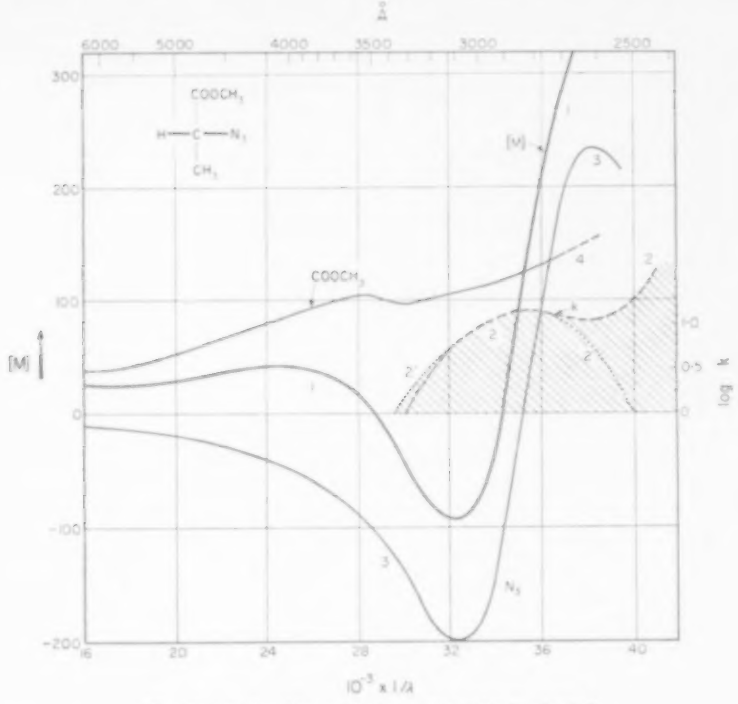


FIG. 3. D(+)-azidopropionic methyl ester in alcohol.

Curve 1: Molecular rotatory power (M) observed.
Curve 2: Molecular absorption coefficient observed.
Curve 2′: N_3-absorption band (with Gaussian error curve shape), used as basis for computing curve 3.
Curve 3: Rotatory contribution of the N_3-absorption band (curve 2 or 2′).
Curve 4: Difference of curve 1 and 3; significance: rotatory contribution from the remaining absorption bands of the molecule, i.e. essentially rotatory contribution of the $COOCH_3$-absorption band.

contribution of the next absorption band which has to be attributed to the $COOCH_3$-group and to the $CON(CH_3)_2$-group of the ester and the dimethylamide. It is noted that the $COOCH_3$ and $CON(CH_3)_2$-group which shows the enormous change in the rotatory contribution (Fig. 4) is the group in the molecule which so to say *personally* undergoes the big change when going from the one to the other of the two molecules compared.

Statements similar to those made for the N_3-absorption band and the $COOCH_3$- and $CON(CH_3)_2$-absorption bands in the azidopropionic ester and dimethylamides have been made experimentally in a number of further more or less analogous cases, e.g. for the halogen absorption bands in α-halogenated acids,[5,8] the phenyl absorption band in mandelic acid and its derivatives, the O-NO absorption bands of nitrites, etc.

We are now going to *transform these findings concerning the Cotton-effects or the rotatory dispersion into findings concerning the vibrating momentum of the absorption bands* and to interpret the regularities by physical considerations concerning the vibrating moment. This is possible because we can state that the origin of the Cotton-effect of a given absorption band is substantially a description of the vibrating electrical momentum associated with the absorption band considered. The main point may again be elucidated in the case of the N_3-absorption band near 2900 Å which gives the pronounced Cotton-effect in azidopropionic acid and dimethylamide, shown in Fig. 1 and 3. An absorption band whose vibrating momentum is isotropic or unidirectionally

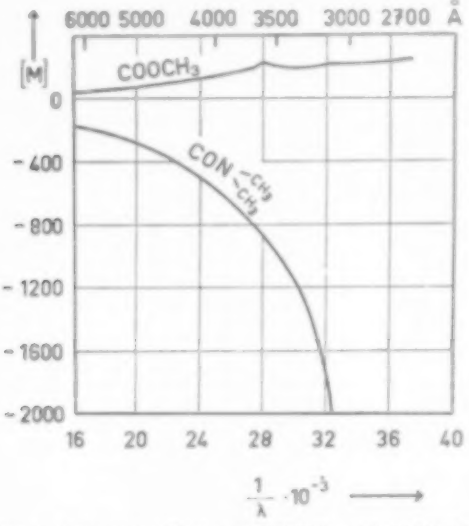


FIG. 4. Approximate rotatory contribution of $COOCH_3$- and $CON(CH_3)_2$-absorption band of of D-configurated azidopropionic ester and dimethylamide (i.e. curves 4 from Fig. 4 and 1).

Mode of vibration of optically active absorption bands.

orientated in the molecule will not be circularly dichroitic. This is the reason why the N_3-absorption band near 2900 Å present in ethyl azide is not optically active though situated at the same wavelength as the N_3-absorption band in azidopropionic acid and dimethylamide. For the N_3-absorption band to become optically active, it is necessary that its vibrating moment will have components non parallel and non complanar relative to each other in distant parts of the molecule.[8-10,13] According to this the vibrating moment of the N_3-absorption band of azidopropionic dimethylamide (point 1 of Fig. 5a) will have some component in the region of the $OCN(CH_3)_2$-group (point 2 of Fig. 5a) non parallel and non complanar to the main vibrating moment located in the N_3 group itself (point 1 of Fig. 5a). It is indeed seen that a system with the mode of vibration shown in Fig. 6 will respond differently to a beam of right hand or left hand circular light, i.e. that it will show circular dichroism and optical rotation.

[13] For a discussion of the vibrating moment in the case of weak absorption bands where the anisotropy factor is, as a rule, much higher than in the case of strong absorption bands (a discussion not contained in ref. 8) see W. Kuhn and K. Bein, *Z. Phys. Chem.* (B) **22**, 406 (1933); see also W. Kuhn, *Angew. Chem.* **68**, 93 (1956); *An. Rev. Phys. Chem.* **9**, 417 (1958).

As far as the participation of the $CON(CH_3)_2$-group in the mode of vibration of the N_3-absorption band is concerned, this participation is mainly produced by the electronic polarizability and the orientation of the polarizability of the group. A vibrating moment existing in the N_3-group (point 1 of Fig. 5) will indeed, due to the existence of a polarizable oriented group in point 2, produce an induced momentum in the latter. A less important[14] second effect is according to the one-electron theory of optical activity[11] based on the electric field produced by the $CON(CH_3)_2$-group in the region of the N_3-group.

We have called *vicinal action* the participation of a neighbouring group e.g. the

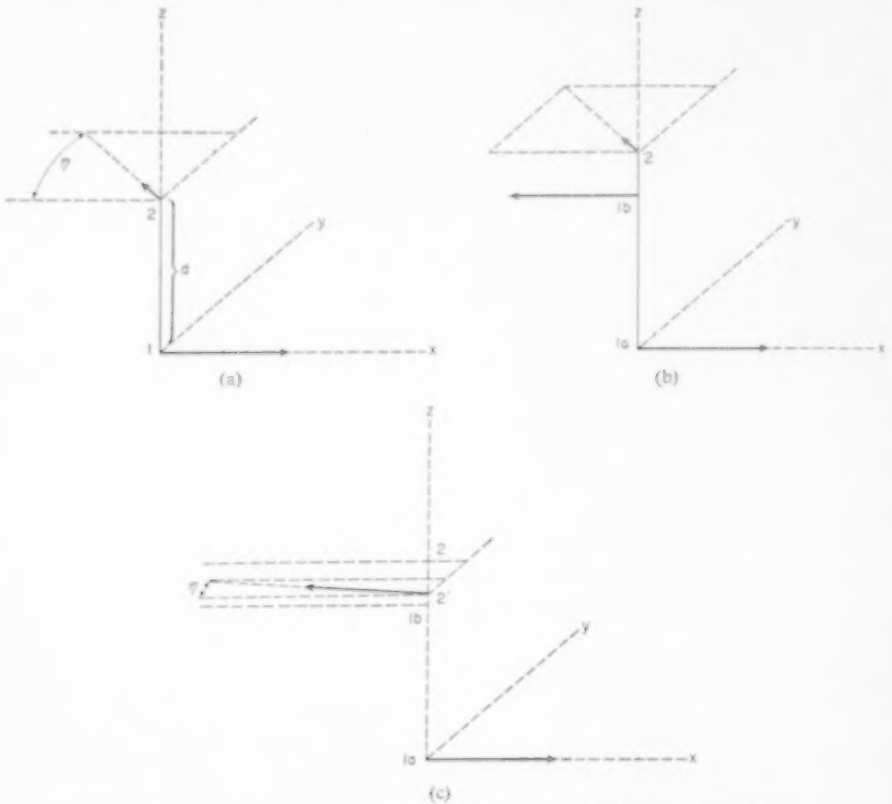


FIG. 5a. A vibrating momentum localized (in a first approximation) in point 1 produces in a polarizable suitably oriented substituent located in point 2 a (small) induced momentum (vibrating with the frequency characteristic for the vibration in point 1). The resulting vibration, having components at angles in various parts of the molecule is optically active. FIGS. 5b and c. Vibrating momentum of *weak* optically active absorption bands. The vibrating moment localized (in a first approximation) in point 1 consists of two nearly equal and nearly antiparallel components in point 1a and 1b (quadrupole moment). The vibration in 1a and 1b produces in a polarizable group in point 2 a (small) induced momentum. The resultant of the components in points 1b and 2 (represented by the arrow in point 2' in Fig. 6c) together with the component 1a in Fig. 6c is a quadrupole moment with nearly antiparallel components of nearly equal magnitude, the angle y in Fig. 6c being very small. Vibrating momenta of this type are shown to give particularly high values of the anisotropy factor[12] (much higher than vibrating momenta of type Fig. 6a). They are however sensitive to small disturbances of y (solvent influence etc.).

[14] W. Kuhn, *Z. Elektrochem.* **56**, 506 (1952).

$CON(CH_3)_2$-group in the vibrating moment of a chromophoric group (e.g. the N_3-group) of the same molecule. As this vicinal action is mainly based on the polarizability and its orientation inside the molecule it is plausible that this action is not extremely sensitive to a small chemical change of the group, say of the $CON(CH_3)_2$-group as long as the general character of the group is maintained. This is exactly what has been observed in the example considered: the transition from the dimethylamide to the methyl ester has left the vicinal action, i.e. the participation of the $CON(CH_3)_2$- or $COOCH_3$-group in the vibrating moment of the N_3-group and thereby the Cotton-effect of the N_3-absorption band nearly unchanged.

The generalization of these statements and findings can be summarized roughly in the following way:

Each substituent of a chemical compound contributes in two ways to the rotatory power.

1° by being a chromophoric group, i.e. possessing absorption bands which become optically active through the vicinal action of the rest of the substituents.

2° by its vicinal action, i.e. by taking part through the electronic polarizability in the mode of vibrations of neighbouring chromophoric groups. (Helping to make those vibrations optically active.)

The mode of vibration of a given absorption band is, in particular in the case of weak absorption bands where the vibrating moment in a quadrupole moment (Figs. 5b and c) very sensitive to a chemical change produced on the chromophoric group directly because it touches the very origin, the first approximation of the vibrating moment of the individual absorption band. This first approximation is an individual property of an individual absorption band of an individual group, a property which varies from one absorption band of the same group to another and very often even varies inside the absorption region of an individual apparently simple absorption band.[15]

The *vicinal action* on the other hand is less sensitive to a chemical change because it is due to the polarizability *which is an average manifestation of the group.*

Two simple cases where the application of these rules is particularly effective may shortly be mentioned.

(a) *Isolated absorption band*

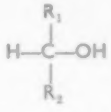
If one isolated absorption band belonging to a specific group is found in the molecule, the Cotton-effect of the absorption band considered can be measured and rules concerning the vicinal action of the surrounding groups be found. Basing on such rules it will be possible to determine the relative configuration of related compounds basing on the fact that similar substituents with similar vicinal function in a similar arrangement will give to the absorption band considered a similar Cotton-effect.

This method which has been illustrated in the case of the N_3-absorption band in azidopropionic dimethylamide and methylester has recently been applied successfully by Djerassi and Klyne to the Cotton-effect of the CO-absorption band of steroids.[16]

[15] E.g. in many of the absorption bands of inorganic complexes, for example in trioxalato–potassium–cobaltiate [W. Kuhn and K. Bein, *Z. Phys. Chem.* (B) **24**, 335 (1934) or in triethylenediaminecobaltibromide J. Mathieu, *J. Chim. Phys.* **33**, 78 (1936)].

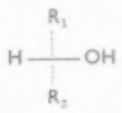
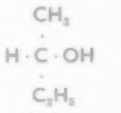
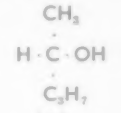
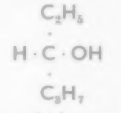
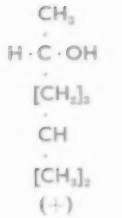
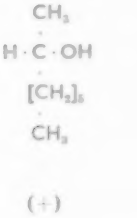
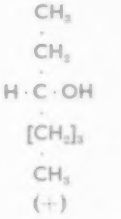
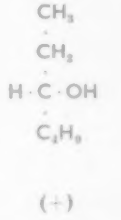
[16] C. Djerassi and W. Klyne, *Chem. & Ind.* 988 (1956); *J. Amer. Chem. Soc.* **79**, 1506 (1957).

If the molecules compared, e.g. aliphatic alcohols, are containing *one* absorption band (in the example the OH-absorption band) in the *near* ultra-violet, the absorption of the rest of the substituents being located in the very far ultra-violet exclusively, the rotatory contribution of the one absorption band (the OH-band) in the near ultra-violet will be so predominant that it determines the sign and the approximate magnitude of the rotatory power in the visible. In this case the vicinal rule explains[8] the fact that aliphatic alcohols of the formula R_1



where R_1 and R_2 are aliphatic alkyl groups and where $R_2 > R_1$ have the same sign of rotatory power in the visible. This is illustrated by the following examples:[8]

Relative configuration and sign of optical rotation of secondary alcohols

			
(+)	(+)	(+)	(+)
$R_1 > R_2$	Methyl-athyl-carbinol	Methyl-n-propyl-carbinol	Aethyl-n-propyl-carbinol
			
(+)	(+)	(+)	(+)

(b) *Several groups giving rotatory contributions of comparable magnitude*

In the majority of cases the molecules will contain several groups giving absorption bands with Cotton-effects in the near ultraviolet in such a way that the rotatory power in the visible cannot be said to be dictated by one specific group or one specific absorption band alone. In the case of D-azido-propionic dimethylamide the contribution of the $OCN(CH_3)_2$-group was seen to be strongly negative, the contribution of the $OC\text{-}OCH_3$-group in the corresponding ester however strongly positive (Fig. 4) with an absolute value which was in the visible greater than the N_3-contribution. This is the reason for a strong shift of the rotatory power in the visible when going from the dimethylamide to the ester in the D-azidopropionic compound. If we compare the D-bromopropionic dimethylamides and esters instead of the azido-compounds, we will expect that the $COOCH_3$-contribution is still positive and the $CON(CH_3)_2$-contribution negative if the vicinal actions of Br and N_3 are similar. In this case the shift when going from the ester to the dimethylamide will again be a shift towards negative values of rotation. A shift is indeed observed in the mercury yellow line from $+92$ to -159 when going from the ester to the dimethylamide of D-α-bromo-propionic acid (Fig. 6, curve 6). An analogous shift is observed in going from the

ester to the dimethylamide in the case of further D-configurated α-substituted acids (Fig. 6). The vicinal rule thus predicts or explains the existence of a shift of the molecular rotation of a specific sign and comparable magnitude when making analogous chemical changes in equally configurated analogous optically active molecules. These

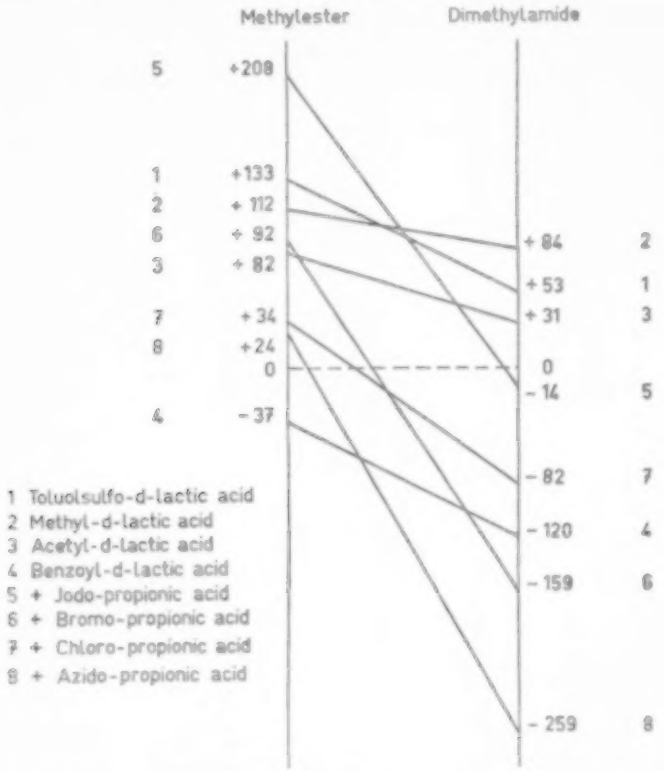


FIG. 6. Rules of shift: A strong shift towards negative values of the molecular rotatory power (for $\lambda = 5780$ Å) is observed in the case of D-configurated α-substituted acids when going from the methylester to the dimethylamide. The data represented in this figure are taken from a table given by K. Freudenberg (ref. 8).

rules of shift, first found by Freudenberg[17] and then explained and corroborated by the vicinal rule brought the complete resolution of contradictions which were brought about by earlier tentative rules for amides and lactones established by C. A. Hudson.[19] They were of great importance for the establishment of the relative configuration especially in the series of the biologically important α-substituted aliphatic acids. The first consistent answer to the question, in which cases Walden inversion takes place and in which cases it does not, resulted from the relative configuration determinations thus obtained.[8,19]

The optical *superposition* and the limitation of its validity[18] are another example

[17] K. Freudenberg, Fr. Brauns and H. Siegel, *Ber. Dtsch. Chem. Ges.* **56**, 193 (1923). K. Freudenberg und L. Markert, *Ibid.* **60**, 2447 (1927); K. Freudenberg and A. Luchs, *Ibid.* **61**, 1083 (1928).
[18] K. Freudenberg and W. Kuhn, *Ber. Dtsch. Chem. Ges.* **64**, 703 (1931).
[19] K. Freudenberg, *Stereochemie* p. 709. Wien (1933); *Heidelb. Akad. Wiss.* No. 14 (1930); *Ber. Dtsch. Chem. Ges.* **66**, 177 (1933); *Monatsh. Chem.* **85**, 537 (1954).

of the application of the vicinal rule to chemical problems and to the description of the relations between optical rotatory power and chemical constitution.

Our insight into all these phenomena is, therefore, a result of the studies of rotatory dispersion, an insight which had and has to be expressed in terms of the vibrating moment of optically active absorption bands. There is no doubt that still more information will be gained through further analysis of the Cotton-effect.

SUMMATION RULE AND RELATIVE IMPORTANCE OF WEAK ABSORPTION BANDS

There is one more point which should be mentioned when discussing optical rotation as a sum of contributions made by various absorption bands of an optically active substance. It is the fact that the sign of the Cotton-effect is necessarily changing

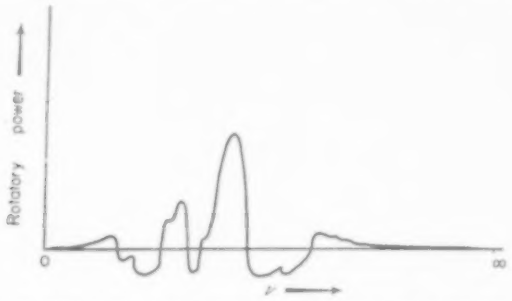


FIG. 7. Optical rotation is a property which, for a given substance, disappears both in the limit of low and high frequencies.

when considering the various absorption bands of a given antipode of an optically active substance. The quantitative treatment shows that the integral

$$\int_0^\infty \frac{\varepsilon_l - \varepsilon_r}{\nu} \mathrm{d}\nu = 0 \qquad \text{or} \qquad \sum f_i g_i \frac{1}{\nu_i} = 0$$

i.e. that the circular dichroism of a given substance, taken over the entire absorption spectrum is zero.[20] For the optical rotation in the visible of a substance possessing absorption bands in the near and far ultra-violet this means that the rotatory contributions are of partly opposite sign; a far-going cancellation of the contributions therefore takes place. The cancellation would be complete if frequency factors were disregarded. The situation is different from the case of ordinary light refraction where the contributions in the visible of the U.V. absorption bands are *all* of the same sign. The peculiar situation in optical activity is also described correctly by the statement that rotatory power is a phenomenon which exactly disappears when going to the limit of both high and low frequencies (Fig. 7).

The existence of this summation rule does by no means impair the validity of the vicinal rules and the validity of the explanations given for the sign of the rotatory power of some groups of substances or the correctness of the rules of shift. Leaving these explanations unchanged it explains however in conjunction with the behaviour of weak absorption bands mentioned the well known sensitivity of the optical rotatory power towards physical and chemical changes. If the rotatory contributions are

[20] W. Kuhn, *Z. Phys. Chem.* (B) **4**, 14 (1929).

nearby cancelling it is obvious indeed that even a minor change, e.g. of the contribution of one weak band may even produce a renewal of the sign of the resulting optical rotation.

It is for this reason in many instances an advantage to base any considerations concerning optical activity as far as possible on the rotatory contributions of single isolated absorption bands.

However the procedure chosen in discussing optical rotatory may be, it will in all cases be essential to be clear about the one point that the investigation of optical rotatory power means the investigation of the vibrating momentum of one or several absorption bands of the molecules, a property which has to be described and defined and investigated in addition to the absorption frequency and intensity and which is of interest to the physicist as well as to the chemist.

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APPLICATION OF ANOMALOUS OPTICAL ROTATORY DISPERSION TO ORGANIC CHEMICAL PROBLEMS*

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ACCORDING to a system of nomenclature which has been introduced recently[1] and which has found general acceptance, an anomalous rotatory dispersion curve is one where a Cotton effect can be demonstrated experimentally. Curves, where this is not possible, are referred to as "plain", irrespective of whether they cross the zero rotation axis. Our nomenclature thus differs from that employed earlier by physical chemists[2] who used a mathematical criterion (Drude equation) and the reasons for this change have already been given elsewhere.[1]

Largely due to experimental difficulties, the overwhelming number of optical rotatory dispersion curves which have been recorded in the literature prior to 1955, have been of the plain type. While such curves—and the Drude equations derived from them—are more useful than the standard $[\alpha]_D$ values usually employed by organic chemists, such plain curves cannot possibly compare in scope with anomalous curves in terms of the information which can be extracted from them. This has been the main thesis of our work in the field of optical rotatory dispersion and the present lecture will be concerned with it.

With relatively few exceptions, most of the important work on optical rotatory dispersion of organic molecules prior to 1955 has been performed by physical chemists, whose motives and interests were usually different from those of organic and biological chemists. It is not surprising, therefore, that until recently, rotatory dispersion measurements, and the conclusions which might be derived from them, have not been employed to any extent for the solution of organic chemical problems. This is in marked contrast to the extensive use of monochromatic polarimetry (rotations measured at 589 mμ, the sodium D line), which has led to a number of generalizations.[3] It should be emphasized that the success of this approach is due to the fact that the sign and magnitude of the rotation at 589 mμ is usually a reflection of the same parameters of the corresponding rotatory dispersion curve. When this is not the case, then calculations of molecular rotation differences are invalid and this usually happens when the dispersion curve crosses the zero rotation axis. A typical example is given in Fig. 1, which contains the dispersion curves[4] of the three isomeric α-(iodophenoxy)-propionic acids (I, II, III). All three acids possess the same absolute configuration, and while all exhibit a plain positive dispersion curve, that of the

* "Optical Rotatory Dispersion Studies XLVII." For paper XLVI see C. Djerassi, K. Undheim, R. C. Sheppard, W. G. Terry and B. Sjöberg, *Acta Chem. Scand.* **15**, In press (1961).

[1] C. Djerassi and W. Klyne, *Proc. Chem. Soc.* 55 (1957). For minor modifications and some additions see Chapter 2 in ref. 10.

[2] E.g., T. M. Lowry, *Trans. Faraday Soc.* **26**, 266 (1930).

[3] For review see W. Klyne in Chapter 3 of *Determination of Organic Structures by Physical Methods* (Edited by E. A. Braude and F. C. Nachod). Academic Press, New York (1955).

[4] B. Sjöberg, *Arkiv. Kemi* **15**, 451 (1960).

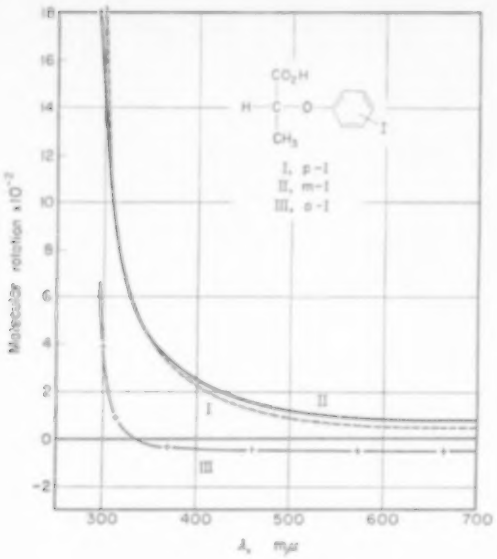
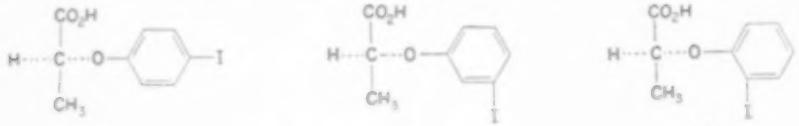


FIG. 1.

o-isomer (III) starts in the visible region on the negative rotation side. Therefore, rotation measurements conducted solely at the sodium D line lead to a negative value for the *o*-isomer (III), while the *p*- and *m*-isomers (I, II) exhibit positive rotations. This case represents only one of many which could be presented to indicate the advantage of rotatory dispersion measurements (irrespective of whether the curves are plain or anomalous) over monochromatic polarimetry.



I II III

We shall now turn to the actual subject matter of the lecture, namely anomalous rotatory dispersion curves and their use in organic chemistry. Prior to 1955, when our first paper[5] appeared, less than fifty rotatory dispersion curves had been recorded in the literature with an experimentally determined Cotton effect. In order to measure this dispersion feature, it is necessary to conduct rotation measurements through the region of absorption and from a practical standpoint, this requires a chromophore absorbing in an accessible region of the spectrum and with a low extinction. For our purpose—correlation of anomalous optical rotatory dispersion with structure and stereochemistry—the carbonyl chromophore appeared to be ideal since it exhibits selective ultraviolet absorption above 270 mμ with very low extinction. The earlier literature[6,7] contains about one dozen examples, largely from the terpene series, of

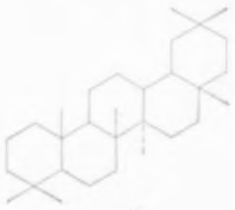
[5] C. Djerassi, E. W. Foltz and A. E. Lippman, *J. Amer. Chem. Soc.* **77**, 4354 (1955).
[6] The literature until 1934 is summarized by one of the pioneers in this field, T. M. Lowry, *Optical Rotatory Power*. Longmans, Green, London (1935).
[7] T. M. Lowry and R. E. Lishmund, *J. Chem. Soc.* 709 (1935); H. S. French and M. Naps, *J. Amer. Chem. Soc.* **58**, 2303 (1936); S. Mitchell and K. Schwarzwald, *J. Chem. Soc.* 889 (1939).

carbonyl compounds where a Cotton effect was measured experimentally. These investigations—notably the classic case of camphor[8] involving both rotatory dispersion and circular dichroism measurements by Kuhn and by Lowry—were prompted by theoretical considerations, and while they demonstrated clearly that the Cotton effect associated with a carbonyl group in an asymmetric environment can be measured experimentally, this information proved to be of no utility for the solution of organic chemical problems and no further studies appear to have been conducted after 1939.[7]

Indeed, what was necessary was to collect a large number of anomalous rotatory dispersion curves of closely related carbonyl-containing substances and to determine whether any correlation existed between the characteristic shape of a Cotton effect curve and some structural or stereochemical feature. We felt that if this could be demonstrated for carbonyl-containing organic substances, interest and research activity with other chromophores would follow automatically. Our work to date has been recorded in thirty-seven articles which have been published since 1955 and they are collected in the appendix to the proceedings of this conference. The most important results have already been reviewed[8,9] and early in 1960, a book has appeared[10] by the author which covers the entire subject as far as applications to organic chemistry are concerned. Therefore, only a few selected examples will be taken to show what information anomalous rotatory dispersion can offer in organic chemistry.

Our initial studies were conducted in the steroid series with carbonyl groups placed in every one of the possible positions of this nucleus (IV). It was possible to show[11] that with few exceptions, a keto group in a given location of the steroid nucleus exhibited a very characteristic dispersion picture which was not affected by the presence of "non-chromophoric" substituents. As a result, anomalous rotatory dispersion can be used as a unique tool to locate a carbonyl group (and hence an alcoholic function which can be oxidized to the ketone) in an unknown steroid. This is not possible by any other physical tool and it is not surprising, therefore, that the steroid field has been the first one in which rotatory dispersion has been accepted as practically a routine adjunct to research. The same statement should also apply to the triterpenoids (e.g. V), where similar structural conclusions could be reached.[12]

IV



V

In principle, a given Cotton effect curve can only be positive or negative, but there are also possible so many variations in shape and amplitude that the scope is enlarged

[8] C. Djerassi, *Bull. Soc. Chim. Fr.* 741 (1957).
[9] C. Djerassi, *Rec. Chem. Progr.* **20**, 101 (1959).
[10] C. Djerassi, *Optical Rotatory Dispersion. Applications to Organic Chemistry*. McGraw-Hill, New York (1960).
[11] Chapter 4 in ref. 10.
[12] Chapter 6 in ref. 10.

enormously and this has made possible the type of structural application indicated above. Clearly, these variations are due to the changes in asymmetric environment in which the carbonyl group finds itself as it is moved around the steroid nucleus. Such an environmental change can also be produced by stereochemical alterations in a given keto steroid. A typical example is shown in Fig. 2 with the rotatory dispersion

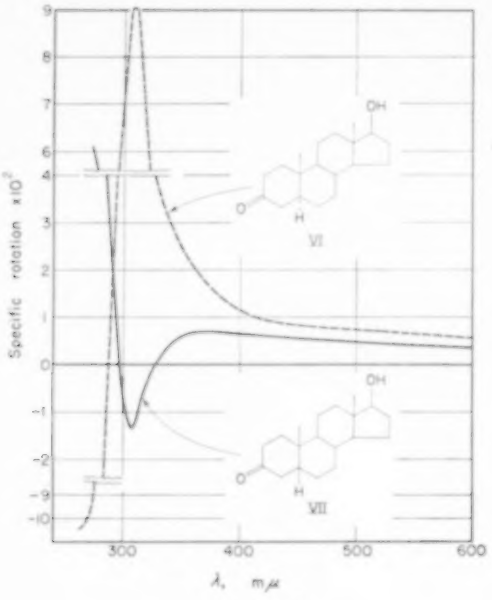
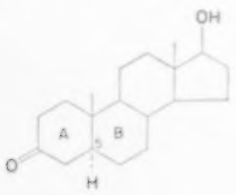
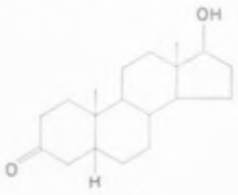


FIG. 2.

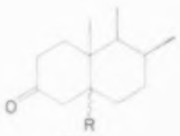
curves of dihydrotestosterone (VI) and its 5β-isomer, VII. These two substances differ only in the nature of the A/B ring juncture, but this stereochemical alteration has profound consequences as far as the rotatory dispersion curves are concerned. These characteristic differences are not altered by the nature of the angular substituent and recent measurements in our Laboratory have shown that, for instance, the C-5 hydrogen atom can be substituted by a variety of other functional groups (VIII) without changing this typical rotatory dispersion pattern. Advantage has been taken of this observation in a number of instances in the recent literature (summarized in refs. 9 and 10) to determine the stereochemistry of the A/B ring juncture in the steroid series. Again, no other physical tool currently available will provide this answer.



VI



VII



VIII

R=CH_3, CN, CO_2CH_3, OH, F, $CONH_2$

When such a change is produced in a keto steroid where the carbonyl group is adjacent to an invertible center, then the rotatory dispersion curve can be used not only for purposes of stereochemical differentiation, but also for kinetic studies. A number of such examples have been reported recently[13] and Fig. 3 offers one such illustration.

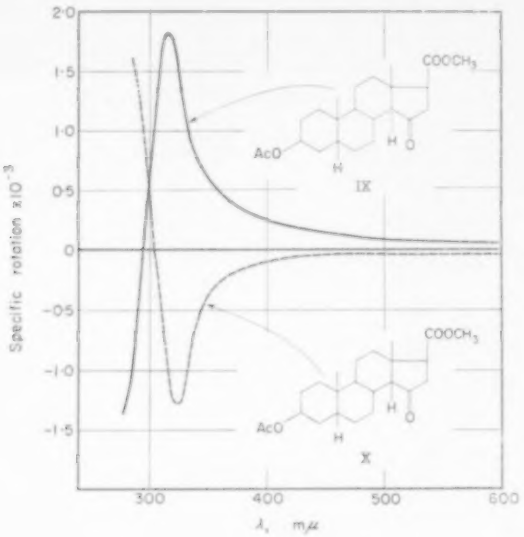
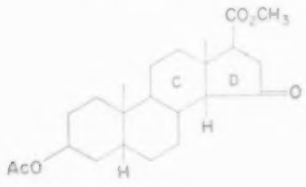
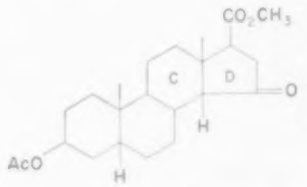


FIG. 3.

The rotatory dispersion curves of the isomeric 15-keto steroids IX and X are of mirror image type and are thus decisive, as far as assignment of configuration of the C/D ring juncture is concerned. Their specific rotations[14] differ by 80° at 589 mμ, while in the 320 mμ region this difference amounts to ca. 3000°. By taking advantage of this enormously increased rotation change in the ultraviolet (near the first extrema of the respective Cotton effects), the kinetics of the base-catalyzed equilibration of such isomers can be determined with 1·0 mg or less of material.



IX



X

The question then arose as to what portion of the molecule was really responsible for these very characteristic rotatory dispersion features. This could be attacked by "dissecting" the steroid molecule in terms of measuring the rotatory dispersion of tricyclic and bicyclic analogs and it was found[15] that qualitatively the dispersion picture is simulated by the bicyclic analog—provided the latter possessed the same

[13] N. L. Allinger, R. B. Hermann and C. Djerassi, *J. Org. Chem.* **25**, 922 (1960).
[14] A. Lardon, H. P. Sigg and T. Reichstein, *Helv. Chim. Acta* **42**, 1457 (1959).
[15] Chapter 5 in ref. 10.

absolute configuration and conformation. This conclusion applies to saturated as well as α,β-unsaturated ketones and has far-reaching consequences as far as the determination of absolute configuration and conformation of organic molecules is concerned.

The subject of absolute configurational determination by this procedure has been covered in earlier reviews[9,16] and two recent examples will suffice. All that is involved is a rotatory dispersion examination of relevant chromophoric systems (one of them representing a reference compound of known absolute configuration) and the process

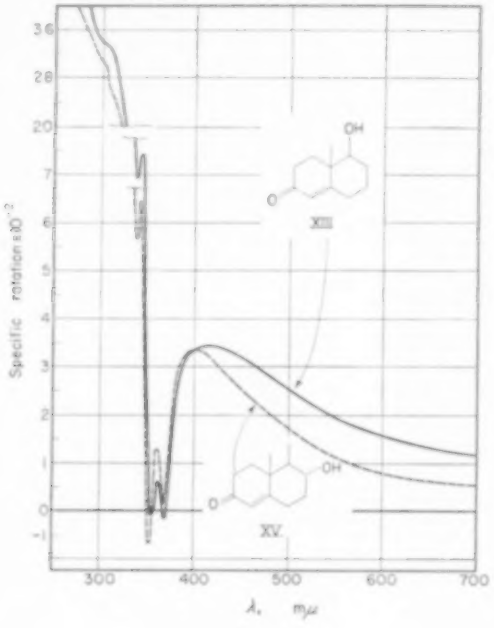
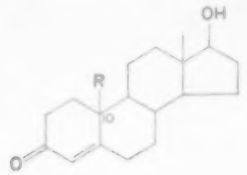


FIG. 4.

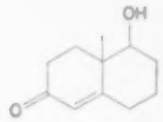
can be illustrated by the recent assignment[17] of absolute configuration to the sesquiterpene petasin (XIV). The absolute configuration of the steroids has been settled by classical means[18] and testosterone (XI) can thus be employed as a reference standard. Alterations in the nature of the angular substituent attached to C-10 have no important effect[19] and 19-nortestosterone (XII), where the angular methyl group is replaced by hydrogen, exhibits substantially the same dispersion curve.[20] Indeed, this observation represents the most cogent evidence presently available for the stereochemistry at C-10 of 19-nor steroids.[21] The bicyclic octalol (XIII) also has the same absolute configuration as can be shown by the coincidence of its rotatory dispersion curve (Fig. 4) with that of testosterone (XI). Returning now to the actual problem at hand—

[16] Chapter 10 in ref. 10.
[17] A. Aebi and C. Djerassi, *Helv. Chim. Acta* **42**, 1785 (1959).
[18] L. F. Fieser and M. Fieser *Steroids* Chap. 10. Reinhold, New York (1959).
[19] The following substituents at C-10 in Δ^4-3-keto steroids have been examined in our laboratory: hydrogen, methyl, hydroxyl, hydroxymethyl, halogen, nitrile, carbalkoxy.
[20] C. Djerassi, R. Riniker and B. Riniker, *J. Amer. Chem. Soc.* **78**, 6377 (1956).
[21] See also C. Djerassi, M. Ehrenstein and G. W. Barber, *Liebigs Ann.* **612**, 93 (1958).

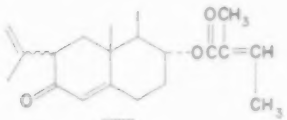
petasin (XIV) can be converted upon treatment with base into des-isopropylidene-petasol (XV). The latter represents a suitable substrate for rotatory dispersion examination and its dispersion curve (Fig. 4) is substantially identical with that of the octalol (XIII) or of testosterone (XI). This coincidental (rather than antipodal) relationship of the two dispersion curves shown in Fig. 4 settles the absolute configuration of petasin (XIV) insofar as its angular substituent is concerned and this rotatory dispersion conclusion[17] has now been confirmed by total synthesis.[22]



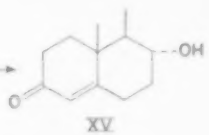
XI R=CH_3
XII R=H



XIII

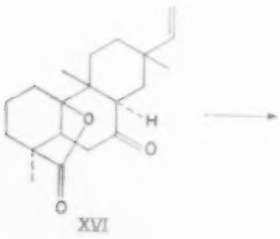


XIV

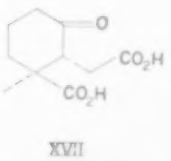


XV

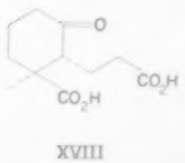
Such an approach has to be used with great caution when dealing with conformationally mobile molecules, such as monocyclic cyclohexanones, since one of the prerequisites of this method is conformational identity of the relevant cyclic systems. A successful application employing substituted cyclohexanones can be cited from the recent determination[23] of the absolute configuration of the mold metabolite rosenonolactone (XVI). This substance can be degraded to (−)-*trans*-2-methyl-2-carboxy-6-oxocyclohexylacetic acid (XVII), whose conformation is almost certainly that chair form in which both acid substituents assume an equatorial orientation. The same statement can also be made of (−)-*trans*-2-methyl-2-carboxy-6-oxocyclohexyl-propionic acid (XVII), whose absolute configuration is known.[24] Since both dibasic acids XVII and XVIII exhibited negative Cotton effect curves of practically identical



XVI



XVII



XVIII

amplitude, one can safely assign identical absolute configurations to them and this conclusion could be verified by further chemical interconversions.[23] Together with

[22] D. Herbst and C. Djerassi, *J. Amer. Chem. Soc.* **82**, 4337 (1960).
[23] W. B. Whalley, G. Green, D. Arigoni, J. J. Britt and C. Djerassi, *J. Amer. Chem. Soc.* **81**, 5520 (1959).
[24] H. H. Inhoffen, S. Schütz, P. Rossberg, O. Berges, K. H. Nordsiek, H. Plenis and E. Höroldt. *Chem. Ber.* **91**, 2626 (1958).

earlier information on the relative stereochemistry of rosenonolactone, the determination of absolute configuration of the cyclohexanone derivative XVII also settles the absolute configuration of the mold metabolite.

The sensitivity of the anomalous rotatory dispersion curve to changes in the asymmetric environment of the optically active chromophore (in this instance the carbonyl group) makes it likely that rotatory dispersion should also be a useful tool for the detection of conformational alterations. This has been found to be the case in

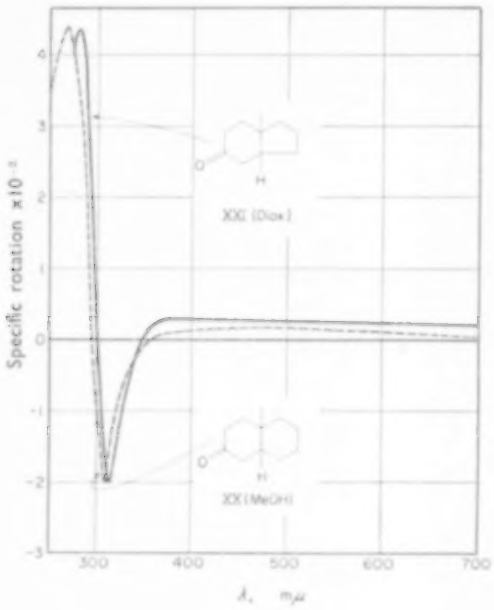


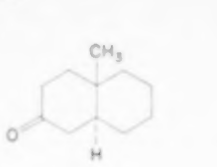
FIG. 5.

numerous instances[25] and a pertinent illustration can be presented from the *cis*-decalone series. Here again, rotatory dispersion has offered information which was not available from any other source.

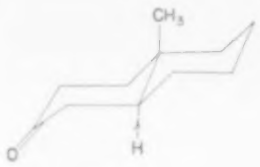
In contrast to *trans*-10-methyl-2-decalone (XIX), whose conformation can be expressed unambiguously in terms of the representation XIXa, *cis*-10-methyl-2-decalone (XX) can exist in two all-chair conformations. The "steroid-like" conformation XXa is obligatory in the steroid series, because of the additional B/C ring juncture, but among decalones, the "non-steroid" conformation XXb is equally plausible and in fact has been favored.[26] In order to attack this problem, optically active *cis*-10-methyl-2-decalone (XX) of known absolute configuration has been synthesized[27] and its rotatory dispersion curve determined. As shown in Fig. 5, this is so similar to that (VII in Fig. 2) of a 3-keto-5β-steroid (VII) that it was concluded[27] that *cis*-10-methyl-2-decalone (XX) exists predominantly in the "steroid-like" conformation XXa. The same situation also applies to *cis*-8-methylhydrindan-5-one

[25] E.g., C. Djerassi, O. Halpern, V. Halpern and B. Riniker, *J. Amer. Chem. Soc.* **80**, 4001 (1958).
[26] W. Klyne, *Experientia* **12**, 119 (1956).
[27] C. Djerassi and D. Marshall, *J. Amer. Chem. Soc.* **80**, 3986 (1958).

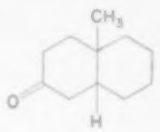
(XXI)[28] and as its rotatory dispersion curve (Fig. 5) is also of the steroid type, the identical conformational conclusion can also be applied to the hydrindanone XXI. Since the corresponding *trans*-8-methylhydrindan-5-one (XXII) had also been synthesized[29] and its rotatory dispersion curve (similar to VI and XIX) measured, there were available two reference curves which proved very useful for settling the outstanding question of the C-5 stereochemistry of B-nor-3-keto steroids (e.g. XXIII).[30]



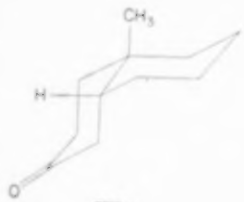
XIX



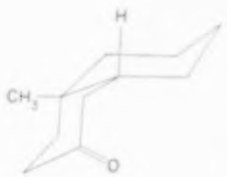
XIX a



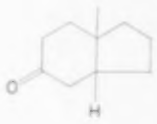
XX



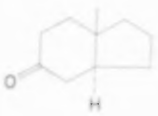
XX a



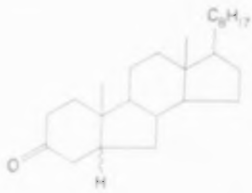
XX b



XXI



XXII



XXIII

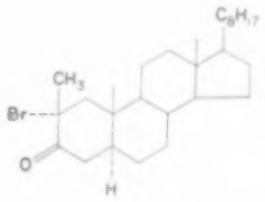
A further and very important advance in conformational and absolute configurational assignments came from a systematic study of the rotatory dispersion behavior of steroidal α-halo ketones.[31] By analyzing the data from a large number of halogenated steroid ketones, where the orientation of the halogen atom had been established independently, an important generalization could be made[32] which is called the axial haloketone rule. This rule states[31,32] that while introduction of equatorial halogen does not change the sign of the Cotton effect of the parent ketone, axial chlorine, bromine or iodine can do so in a predictable manner. Briefly, if the observer looks down the O=C axis of a cyclohexanone as indicated in expression XXIV and the axial halogen atom is to the right (X = halogen), a positive Cotton effect will

[28] H. Conroy and E. Cohen, *J. Org. Chem.* **23**, 616 (1958); W. Acklin and V. Prelog, *Helv. Chim. Acta* **42**, 1239 (1959).
[29] C. Djerassi, D. Marshall and T. Nakano, *J. Amer. Chem. Soc.* **80**, 4853 (1958).
[30] T. Goto and L. F. Fieser, *J. Amer. Chem. Soc.* **81**, 2276 (1959); G. H. R. Summers, *J. Chem. Soc.* 2908 (1959); W. G. Dauben, G. A. Boswell and G. H. Berezin, *J. Amer. Chem. Soc.* **81**, 6082 (1959).
[31] Chapter 9 in ref. 10.
[32] C. Djerassi and W. Klyne, *J. Amer. Chem. Soc.* **79**, 1506 (1957); C. Djerassi, J. Osiecki, R. Riniker and B. Riniker, *Ibid.* **80**, 1216 (1958).

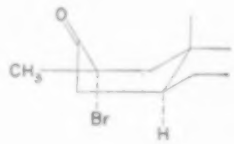
be obtained, while a negative one will be exhibited if the halogen atom is to the left (X′=halogen). An obvious corollary of the axial haloketone rule is that if the absolute configuration of the α-haloketone is known, its conformation can be predicted from the observed Cotton effect, while conversely, an absolute configurational assignment can be made if the conformation is known. From the various applications[9,31] of the axial haloketone rule, two merit special mention.

The first refers to an illustration from the steroid series; the negative Cotton effect of 2α-bromo-2β-methylcholestan-3-one (XXV) represented the first indication[33] that this type of steroid exists in the boat conformation (XXVa) and this conclusion could then be supported[33] by chemical and spectroscopic evidence.

XXIV



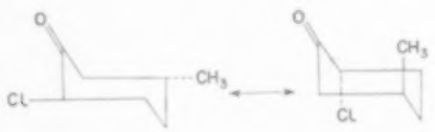
XXV



XXVa

A second example shows how the axial haloketone rule could be used to demonstrate[34] conformational mobility in *trans*-2-chloro-5-methylcyclohexanone. The rule predicts a positive Cotton effect for conformation XXVI (since the chlorine-free parent ketone shows a positive Cotton effect) and a negative one for conformation XXVII. As demonstrated in Fig. 6, the substance gives a negative Cotton effect in octane, while this is altered to a positive one in methanol. This is in accord with the generalization[35] that an increased proportion of the equatorial α-halo ketone (XXVI) can be expected in a polar medium (e.g. methanol) at the expense of the axial isomer (XXVII) which is favored in a non-polar solvent (e.g. octane). Full confirmation of these views could be presented when it was found[36] that the rotatory dispersion curve of *trans*-2-bromo-5-t-butylcyclohexanone (XXVIII) remained completely unaltered in octane or methanol—the positive Cotton effect demonstrating that the substance is "fixed" in the conformation XXVIII, by the anchoring effect of the bulky t-butyl group which requires an equatorial orientation.

The axial α-haloketone rule appears also to be applicable[37] to certain α-bromocyclopentanones, which suggests that rotatory dispersion may offer some useful



XXVI XXVII XXVIII

[33] C. Djerassi, N. Finch and R. Mauli, *J. Amer. Chem. Soc.* **81**, 4997 (1959).
[34] C. Djerassi and L. E. Geller, *Tetrahedron* **3**, 319 (1958); C. Djerassi, L. E. Geller and E. J. Eisenbraun, *J. Org. Chem.* **25**, 1 (1960).
[35] J. Allinger and N. L. Allinger, *Tetrahedron* **2**, 64 (1958).
[36] C. Djerassi, E. J. Warawa, R. E. Wolff and E. J. Eisenbraun, *J. Org. Chem.* **25**, 917 (1960).

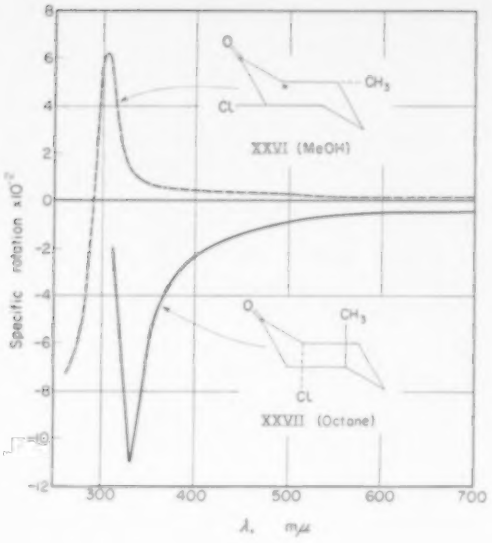


FIG. 6.

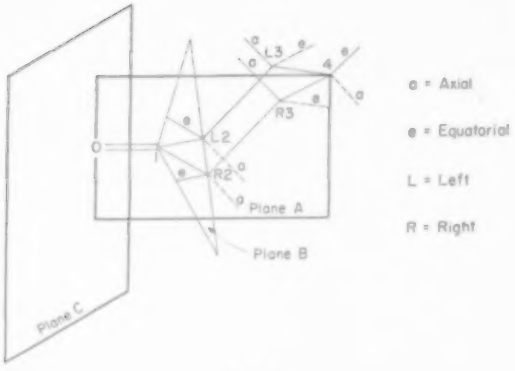


FIG. 7.

contribution to the as yet open problem of the conformation of cyclopentanone.

Recent theoretical studies by Moffitt and Moscowitz[38] on the ultraviolet light absorption of the carbonyl chromophore in an asymmetric environment has led to a generalization, called the octant rule[39] and its applicability to organic chemical problems has already been discussed.[40] The theoretical arguments will be discussed by Dr. Moscowitz[38] and for our purposes, it should suffice that we are dividing the cyclohexanone model as shown in Fig. 7 by three planes corresponding to the nodal and symmetry planes of the 290 mμ transition of the carbonyl chromophore. These three planes create eight octants and the presence of substituents in each octant is given a qualitative rotational contribution. Substituents in the planes (substituents at C-4

[37] J. Fishman and C. Djerassi, *Experientia* **16**, 138 (1960).
[38] To be discussed by Dr. Albert Moscowitz in the succeeding lecture. See also chapter 12 (by A. Moscowitz) in ref. 10.
[39] W. Moffitt, A. Moscowitz, R. B. Woodward, W. Klyne and C. Djerassi, in preparation.
[40] Chapter 13 in ref. 10

as well as equatorial groups attached to carbon atoms L2 and R2 in Fig. 7) are assumed to make no important contribution, while the axial substituents adjacent to the carbonyl group (attached to L2 and R2) behave exactly as expressed above in the axial haloketone rule. Therefore, so far the axial haloketone and octant rules are identical except that the former was limited to halogen atoms while the latter attributes such rotational effects to other substituents as well (e.g. alkyl groups). A further consequence of the octant rule is that it also takes into consideration substituents at the two β-carbon atoms (L3 and R3): the axial and equatorial substituents at carbon atom R3 are assumed to make a negative rotatory contribution while a positive one is attributed to the axial and equatorial groups attached to L3.

In certain cyclohexanones, substituents may also be present to the left of plane C (Fig. 7) in one of the four near octants, and groups in these near octants are assumed to make a rotational contribution opposite in sign to that of a substituent in the corresponding far octant. In terms of application, the octant rule covers the same area as the axial haloketone rule except that its scope is much broader since it is not limited to halogen substituents. When an axial chlorine, bromine or iodine atom is present in the α-position, then for qualitative purposes alkyl substituents at other carbon atoms in the cyclohexanone can usually be ignored because of the halogen's much more powerful rotatory contribution associated with its much higher atomic refractivity,[41] and the simpler axial haloketone rule may be employed.

The full effectiveness of the octant rule will only become apparent when quantitative parameters may be assigned to different types of substituents at the various

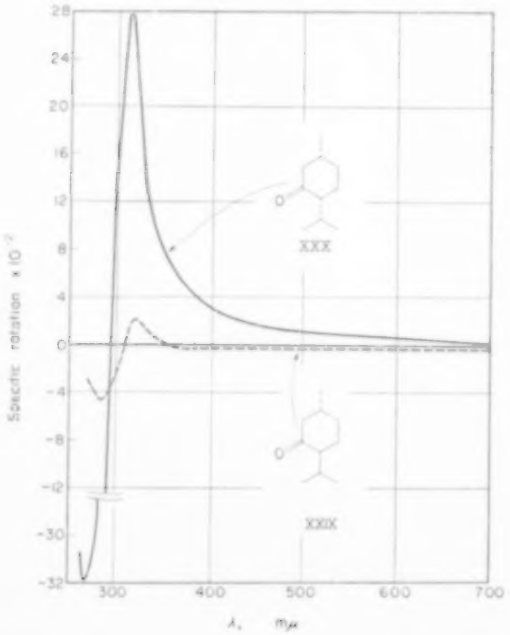
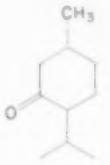


FIG. 8.

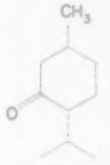
[41] See for instance J. H. Brewster, *J. Amer. Chem. Soc.* **81**, 5475 (1959), Table I.

carbon atoms of such cyclohexanones. Many of the Cotton effect curves measured in our laboratory are now being analyzed in terms of reduced rotational strength[38] by a procedure proposed by Dr. Moscowitz and this will be covered briefly in his lecture. (At the present qualitative stage, the octant rule has served to confirm conformational conclusions reached earlier[27] by direct visual comparisons of rotatory dispersion curves e.g. conformation XXa rather than XXb for *cis*-10-methyl-2-decalone (XX)), to establish certain absolute configurations[40] and most importantly, to pose certain problems in conformational analysis.

For instance, inversion of the isopropyl group of (−)-menthone (XXIX) leads to (+)-isomenthone (XXX). Of the two possible chair conformations for XXX, the octant rule would predict a strong positive Cotton effect for XXXa and a negative one for XXXb and *a priori*, the latter might be expected to predominate. However, the strong positive Cotton effect (Fig. 8) of (+)-isomenthone (XXX) suggests that conformation XXXa is the preferred one and this conclusion raises some interesting questions with respect to the "2-alkyl ketone effect".[26] Extensive work on the synthesis and rotatory dispersion of such alkylated cyclohexanones is now in progress in our laboratory[42] and the predominance of a given conformational isomer can in many instances be predicted by the octant rule.

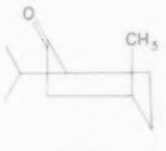


XXIX



XXX

XXX a



XXX b

A final rotatory dispersion approach, which is of some significance[43] in conformational analytical work, concerns the detection and extent of ketal formation. Since ketals would not be expected to show any Cotton effect in the 300 mμ region because of lack of absorption, the extent to which the amplitude of the Cotton effect associated with a given carbonyl group is diminished under ketal-forming conditions, would represent a quantitative indication of such a derivative. In fact, this can be accomplished[44] very readily by measuring the rotatory dispersion curve of a ketone in methanol followed by the addition of hydrochloric acid and repetition of the dispersion measurement. Since mineral acid promotes ketal formation, the reduction in the amplitude of the Cotton effect affords a direct measure of the extent of ketal production and since this is very sensitive to steric factors, there is available still

[42] Unpublished work with J. Osiecki and E. J. Eisenbraun.
[43] Chapter 11 in ref. 10.
[44] C. Djerassi, L. A. Mitscher and B. J. Mitscher, *J. Amer. Chem. Soc.* **81**, 947 (1959).

another tool for determining such steric interactions on a microscale. The applications of this approach to structural and stereochemical problems in organic chemistry have already been reviewed recently,[9,43] and it will only be necessary to state at this time that ketal formation is inhibited by the introduction of alkyl substituents adjacent to the carbonyl group as well as by the generation of new 1,3-diaxial interactions. The operation of these effects can be demonstrated with *trans*-1,9-dimethyldecaline-2,6-dione (XXXI), which has recently been synthesized.[22] The isolated 6-keto group exhibits a positive Cotton effect (similar to XIX), while the 2-keto function shows a very strong negative Cotton effect. The latter is more powerful and

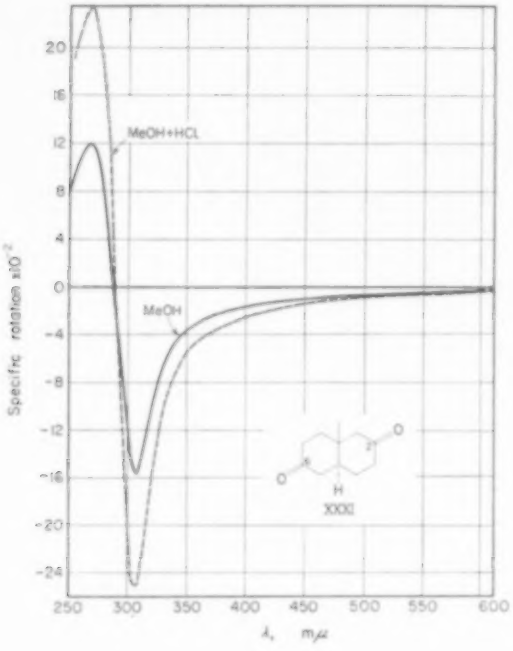
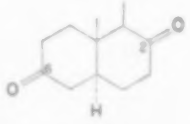


FIG. 9.

as a consequence the 2,6-diketone XXXI possesses a negative Cotton effect curve (Fig. 9). In methanol solution in the presence of hydrochloric acid, the ketone at C-6 is largely converted into its ketal, because of the unhindered nature of the carbonyl group and the absence of any serious steric hindrance in the ketal. On the other hand, the 2-keto group does not react since it is flanked by a methyl group, which greatly reduces ketal formation and even more importantly, it has an axial methyl group two carbon atoms removed. Consequently, in the ketal, there would be set up a new 1,3-diaxial interaction and both these factors eliminate any possibility



XXXI

of ketal production. As a result, the positive contribution of the 6-keto function is removed in the presence of hydrochloric acid in the form of its "transparent" ketal and the Cotton effect (Fig. 9) is now much more negative since it reflects only the strong negative contribution of the carbonyl group at C-2.

The above discussion of the various ramifications and uses to which the anomalous rotatory behavior of only one chromophore—the carbonyl chromophore—can be put serves to show the enormous scope associated with anomalous rotatory dispersion measurements as compared to plain dispersion curves, let alone monochromatic polarimetry. These results have already led to a wide acceptance of rotatory dispersion as a tool in organic chemistry and they have stimulated the examination of other chromophores in a similar manner. Indeed, it is this area which now forms the bulk of current investigations under way in our laboratory and preliminary results with several chromophores (e.g. thiones, nitroalkanes, disulfides, etc.) have already been reported.[45]

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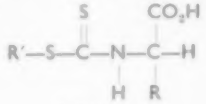
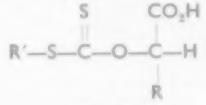
At this time, mention will be made of only one such series, namely the dithiocarbamates, which appear to be of particular utility in the α-amino acid and peptide fields.[46] Briefly, we found that dithiocarbamates of type XXXII show very strong Cotton effects in the 350–400 mμ region and that the sign of the Cotton effect is directly related to the configuration of the asymmetric center. Thus all such derivatives of L-α-amino acids will exhibit positive Cotton effect curves while negative ones will be shown by members of the D-series. This offers an extremely simple device for determining the absolute configuration of such a center and this appears to be also applicable to terminal amino acids in peptides, irrespective of the configuration of the asymmetric center to which the amide nitrogen is linked. Thus, N-dithiocarbomethoxy L-alanyl-L-alanine and N-dithiocarbomethoxy L-alanyl-D-alanine give positive Cotton effect curves of nearly identical amplitude. A similar behavior was noted[46] with xanthates of α-hydroxy acids (XXXIII), membership in the L-series carrying with it a positive Cotton effect. This affords a very simple alternative to the frequently quite vexing problem[47] of assigning absolute configurations to α-hydroxy acids—the sign of rotation at the sodium D line offering only little information (e.g. (+)-lactic acid and (−)-malic acid, both belonging to the L-series).



XXXII



XXXIII

These last examples demonstrate that the choice of a proper chromophore can convert an optically active substance possessing a plain dispersion curve into a derivative with an anomalous one with all of its inherent advantages. Work currently under way in our laboratory[48] has shown that even the unpromising mono-olefins (which absorb in a region which is currently quite inaccessible as far as rotatory dispersion measurements are concerned) can be converted into complexes with Cotton effect curves above 300 mμ.

[45] Chapter 14 in ref. 10.
[46] B. Sjöberg, A. Fredga and C. Djerassi, *J. Amer. Chem. Soc.* **81**, 5002 (1959).
[47] See for instance the summary by K. Mislow, *J. Amer. Chem. Soc.* **73**, 3954 (1951) concerning the absolute configuration of (+)-mandelic acid.
[48] E. Bunnenberg and C. Djerassi, *J. Amer. Chem. Soc.* **82**, 5953 (1960)

It is my feeling that a great deal of very fruitful work still remains to be done in the area of anomalous dispersion curves and that as additional chromophores are investigated, the applications of rotatory dispersion to organic chemistry will multiply manifold. In this connection, it should be emphasized that improvements in instrumentation should not be neglected. As spectropolarimeters are developed with greater penetration into the ultraviolet, more and more plain dispersion curves will become anomalous and to that extent will increase the scope of this tool. Finally, it would be highly desirable if rotatory dispersion studies would be supplemented or even substituted by direct determinations of circular dichroism. A commercially available instrument permitting rapid measurement of circular dichroism would represent a great advance in this field.

Acknowledgement—My deep gratitude is due to those students of mine who have collaborated with me in developing rotatory dispersion measurements as a tool for the solution of organic problems. Their names are listed in the bibliography in the appendix, which contains references to the thirty-seven papers which have so far appeared from our laboratory in the rotatory dispersion field.

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OPTICAL ROTATORY DISPERSION

SOME APPLICATIONS TO STRUCTURAL AND STEREOCHEMICAL PROBLEMS IN ORGANIC CHEMISTRY

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THIS paper deals with work that has been carried out in parallel with the studies of Djerassi (discussed in the previous paper[1]) although starting at a much later date. The first half of the paper is concerned with ketones; in this field our thinking has been guided by the Octant Rule, ever since the Harvard discussions of July and August, 1958.[2] The theoretical and practical aspects of the Octant Rule have already been discussed here by Moscowitz[3] and Djerassi[1], and the present paper deals with some applications which have specially interested me. In this treatment many experimental data are taken from Djerassi's papers—and some are from published and unpublished work by Prof. G. Ourisson of Strasbourg.

The second half of the paper deals with some, as yet rather unrewarding, studies on plain curves (for nomenclature[1])—i.e. on compounds which have no absorption band within the range of wavelengths studied.

Ketones

1. *General*

In this paper the sign and amplitude of the Cotton effect will be considered as the principal characteristics of the dispersion curves of saturated ketones. All rotations are given as molecular rotations [ϕ]; amplitudes (a) are given as the algebraic difference between [ϕ] for peak and trough—divided by 100 for convenience.

In some cases the extremum (peak or trough) of lower wavelength could not be reached; in such cases the 'amplitude' given is the algebraic difference between the peak (or trough) of higher wavelength and the rotation for the lowest wavelength that could be measured. The true amplitude may therefore be greater than the value given, which is distinguished by an exclamation mark (!).

2. *Cotton effect. Lack of influence of remote substituents*

Many examples have been given in Djerassi's work (reviews [5–8]) of the fact that substituents remote from a carbonyl group have little influence on the amplitude of

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[1] C. Djerassi, *Tetrahedron*, **13**, 13 (1961).
[2] W. Moffitt, R. B. Woodward, A. Moscowitz, W. Klyne and C. Djerassi, in preparation.
[3] A. Moscowitz, *Tetrahedron* **13**, 48 (1961).
[4] C. Djerassi and W. Klyne, *Proc. Chem. Soc.* 55 (1957).
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[8] W. Klyne and A. C. Parker, *Physical Methods of Organic Chemistry* (Edited by A. Weissberger) 3rd Ed. Interscience, New York (1960).

its Cotton effect. We have, in the course of other work, assembled data for three series of steroid ketones (Formulae I, II, III), which enable us to confirm these findings, and make a roughly quantitative statement (see Table 1).

For substituents on a rigid skeleton such as that of the steroids, the introduction

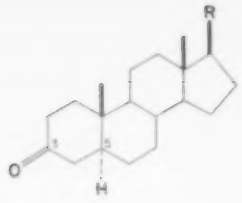
TABLE 1. STEROID KETONES. ABSENCE OF EFFECT BY REMOTE SUBSTITUENTS ON AMPLITUDE OF DISPERSION CURVES*†

Compound	Formula	Amplitude
	3-Oxo-5α-steroids (Formula I)	
5α-Androstan-3-one	R = H	+52
5α-Pregnan-3-one	R = Et	+53
5α-Pregnan-3-one,20β-hydroxy	R = CHOH.Me	+57
5α-Cholestan-3-one	R = C_8H_{17}	+56[a]
5α-Stigmastan-3-one	R = $C_{10}H_{21}$	+54
5α-Spirostan-3-one	R = $C_8H_{14}O_2$	+61[b]
3-Oxo-5α-etianate, methyl	R = CO_2Me	+50
	3-Oxo-5β-steroids (Formula II)	
5β-Androstan-3-one	R = H	−11
5β-Androstan,17α-hydroxy	R = OH(α)	−17
5β-Androstan,17β-hydroxy	R = OH(β)	−22
5β-Androstan,17β-acetoxy	R = OAc(β)	−20
5β-Cholestan-3-one	R = C_8H_{17}	−25
3-Oxo-5β-cholanate, methyl	R = $CHMe.[CH_2]_2.CO_2Me$	−19!
	17-Oxosteroids (Formula III)	
5α-Androstan-17-one		+145
5α-Androstan,3α-hydroxy		+144
5α-Androstan,3β-hydroxy		+148
5α-Androstan,3α-thiol		+139
5α-Androstan,3β-thiol		+136
5β-Androstan-17-one		+149
5β-Androstan-3α-hydroxy		+145
5β-Androstan-3β-hydroxy		+142
Androst-5-en-17-one,3β-hydroxy		+143

* Solvent, methanol, unless otherwise indicated.
† See Djerassi *et al.*, *J. Amer. Chem. Soc.* **78**, 3163, 3761, 6362 (1956).
[a] Duplicate determination, +60
[b] +65 in $CHCl_3$

of alkyl, hydroxyl or even benzoyloxy groups at distances of 7 or 8 Å from a carbonyl group makes a barely significant difference to the amplitude of the Cotton effect.

Substituents closer to the carbonyl group, e.g. 17- or 20-substituents in an 11-oxosteroid (IV), do make a significant difference to the Cotton effect of the carbonyl group, as might be expected; studies are in progress jointly with Professor E. R. H. Jones (Oxford), but are as yet incomplete; two compounds have been found (3α,20β-diacetoxy-5β-pregnan-11-one and methyl 3α-acetoxy-11-oxo-5β-etianate) in which the Cotton effect is almost suppressed. Until we have studied a more extended series of



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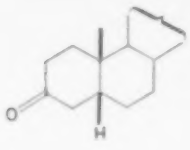

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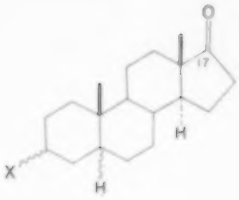

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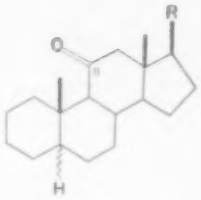
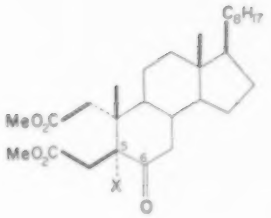


IV



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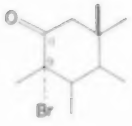

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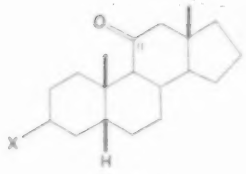

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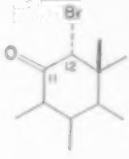

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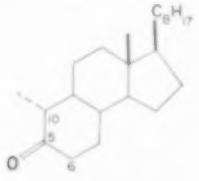


IX

compounds, speculations regarding the reasons for these abnormal curves are premature.

3. *Use of the Octant Rule for configurational allotments*

The following examples illustrate the use of the Octant Rule (or its forerunner, of more restricted scope, the axial α-haloketone rule[9]).

[9] C. Djerassi and W. Klyne, *J. Amer. Chem. Soc.* **79**, 1506 (1957); C. Djerassi, J. Osiecki, R. Riniker and B. Riniker *Ibid.* **80**, 1216 (1958).

Summers[10] in Swansea obtained by bromination of the 6-oxo-2:3-seco-steroid (V, X = H) a bromocompound which showed a very large negative Cotton effect; this indicates conclusively that the bromine is *axial* and in the 5α-position (V; X = Br). Amplitudes (V, X = H) −63; (V, X = Br) −368.

Jones and his colleagues[11] in Oxford in their studies of 11-oxosteroids (VII) obtained three pairs of α-bromo derivatives; the large amplitude-contributions of the bromine substituents (one positive and one negative in each pair) show that these

TABLE 2. 9α- AND 12α-BROMO-11-OXOSTEROIDS*†

Compound	Amplitudes (*a*)		
	11-CO without α-substituent	9α-Br-11-CO	12α-Br-11-CO
5β-Androstan-11-one,3α-hydroxy or 3α-acetoxy	−13	−204	−206, −214
5β-Androstan-11-one, 3-ethylenedioxy	−11	−247	−242
5β-Androstan-3,11-dione	−15	−222	−199

* Solvent, methanol
† See Djerassi *et al.*[9]

are 9α-bromo and 12α-bromo compounds (VI and VIII) respectively. See Table 2, and compare previous results by Djerassi *et al.*[9]

Similar use of rotatory dispersion curves for configurational allotments has been made by Jones *et al.*[12] in their studies on steroids lacking ring A (de-A-cholestan-5-ones; IX). The reference compound without a bromine substituent (IX) was measured by Djerassi (*a*, −76); three different monobromo compounds gave the following results in our hands.

6α-bromo (equatorial) *a*, −88
6β-bromo (axial) *a*, −332
10β-bromo (axial) *a*, +205!

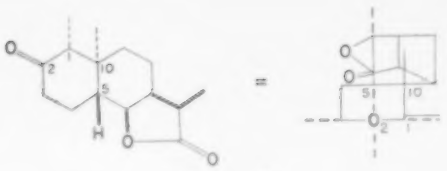
Barton and Gilham[13] obtained from lumisantonin a *trans*-2-decalone, the negative Cotton effect of which (*a*, −79) showed that it was of type (X)—enantiomeric in type to a 2-oxosteroid.

Prelog and Smith[14] deduced the (9*R*) absolute configuration of a 10-hydroxy-*trans*-2-decalone (XI) from its negative Cotton effect (*a*, −67).

4. 2-*Hydroxycyclohexanones and the Octant Rule*

Hitherto the various authors interested in the Octant Rule have refrained from bringing α-ketols of the 2-hydroxycyclohexanone type into consideration—perhaps because of a suspicion that hydrogen-bonding, solvation or other factors might

[10] G. H. R. Summers, *J. Chem. Soc.* In preparation.
[11] E. R. H. Jones *et al.* Unpublished work.
[12] E. R. H. Jones *et al.* Unpublished work.
[13] D. H. R. Barton and P. T. Gilham, *Proc. Chem. Soc.* 391 (1959).
[14] V. Prelog and H. E. Smith, *Helv. Chim. Acta* **42**, 2624 (1959).



X

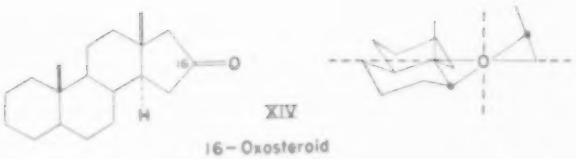
XI

XII

XII A

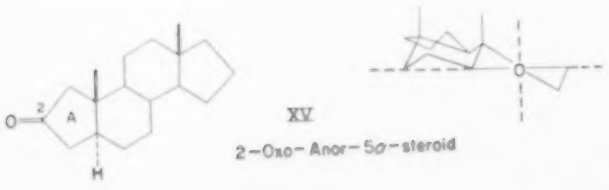
C-4,5,6,7 lie approximately behind
C-3,9,8,1 respectively

XIII



XIV

16-Oxosteroid



XV

2-Oxo-Anor-5α-steroid

complicate the situation. It now seems, however, appropriate to survey the available data—chiefly from Djerassi's work,[15] with some additions of our own.

A very condensed survey is given in Table 3; from this the following groups have been omitted for the reasons given.

(*a*) cyclopentanone ketols—e.g. 16,17-ketols—because the shape of the five membered ring is inadequately known (cf. Fishman and Djerassi,[16] also later pp. 5–10).

(*b*) some D-homosteroid ketols—17,17*a*-ketols—since certain simple ketones of this series behave abnormally.

(*c*) 14β-steroids (i.e. C,D-*cis* fused steroids)—because the conformation of the C ring is not definitely known.

Table 3 shows that for the remaining steroid α-ketols it is generally true that (*a*) *equatorial* hydroxyl or acyloxy substituents have little influence on the Cotton effect; (*b*) *axial* hydroxyl or acyloxy substituents in rings A and B make large contributions to the Cotton effects of the *same* sign as chlorine or bromine in the same positions; (*c*) *axial* hydroxy or acyloxy substituents in ring C make large contributions to the Cotton effect of the *opposite* sign to those given by chlorine and bromine in the same position.

This anomaly is stated without any suggestion of a possible solution; it may be that a modified chair conformation of ring C is partly responsible.

5. *Hexahydroindanones and related ketones*

It has been clear for some months that difficulties would arise in applying to alicyclic ketones in which the carbonyl group is in a five-membered ring, the Octant Rule which has been so successful in summarizing the results for the cyclohexanones and related ketones.

Recent findings in the hexahydroindanone series have led me to suggest that the skewed nature of the five-membered ring itself exerts a strong influence on the symmetry of the carbonyl group here—in contrast to the six-membered ring, where the ring itself is perfectly symmetrical about the carbonyl group, and asymmetry is due to the second-order effects of substituents.

Lack of precise knowledge about the shapes of five-membered rings[16–20] precludes any detailed quantitative treatment, but the following notes sketch a rough picture which seems to cover the major features. (Most data are taken from Djerassi's papers; refs. see Table 4.)

trans-*Hexahydroindan-2-ones*. These compounds of general formula (XII) are distinguished by the very large amplitudes of their Cotton effects (greater than 200). Until recently they have been represented by the compound carrying a methyl group at one ring junction (XII, R = Me), and extended analogues of this (16-oxo- and A-nor-2-oxo-steroids).

Recently Miss P. Bourn (I.C.I. Ltd., Welwyn) has prepared the non-methylated

[15] C. Djerassi, O. Halpern, V. Halpern, O. Schindler and C. Tamm, *Helv. Chim. Acta* **41**, 250 (1958).
[16] J. Fishman and C. Djerassi, *Experientia* **16**, 138 (1960). (I am indebted to Prof. C. Djerassi for a copy of this paper before publication.)
[17] C. G. Le Fevre and R. J. W. Le Fevre, *J. Chem. Soc.* 3549 (1956).
[18] K. S. Pitzer and W. E. Donath, *J. Amer. Chem. Soc.* **81**, 3213 (1959).
[19] F. V. Brutcher, T. Roberts, S. J. Barr and N. Pearson, *J. Amer. Chem. Soc.* **81**, 4915 (1959).
[20] C. W. Shoppee, R. H. Jenkins and G. H. R. Summers, *J. Chem. Soc.* 3048 (1959).

TABLE 3. α-KETOLS AND THEIR ACETATES*†

No reference is made to different substituents at C-3 for 11- and 12-oxo-compounds.

Position of keto group	Series	α-Substituents OR	Amplitude (*a*)	Contribution of OR (Δa)	Reference and source
3	5α-Cholestane	none	+58		*a*
		2α-OAc (eq.)	+66	+8	*a, p*
		2β-OAc (ax.)	+116	+58	*a, p*
3	4,4,Dimethyl-5α-cholestane	none	−12		*b*
		2α-OAc (eq.)	+38	+50	*a, q*
		2β-OAc (ax.)§	+124	+136	*a, q*
4	5α-Cholestane	none	−95		*c*
		5α-OH (ax.)	−45	+50	*d*
6	5α-Cholestane	none	−78		*c*
		5α-OH (ax.)	−110	−32	*d*
		5α-OAc (ax.)	−89	−11	*d*
11	5α-Spirostan	none	+43D		
		12β-OH (eq.)	+44!D	+1!	*d*
		12β-OAc (eq.)	+42!D	−1!	*d*
		12α-OH (ax.)	+42D	−1	*d*
		12α-OAc (ax.)	+88D	+45	*d*
11	5β-Cholanate	none			
		12β-OH (eq.)	+14!		*d*
		12β-OAc (eq.)	+28		*d*
		12α-OAc (ax.)	+73		*d*
11	5α-Ergostane	none	+19		*a, r*
		9α-OH (ax.)	+68	+49	*a, r*
11	5α-Ergostane	none	+12		*c*
		12α-OAc (ax.)	+85	+73	*a, r*
11	5β-Etianate	none	+10		*a, s*
		12α-OH (ax.)	+40	+30	*a, s*
		12α-OAc (ax.)	+44	+34	*a, s*
		12β-OAc (eq.)	+17!D	+7!	*d*
12	5α-Spirostan	none			
		11α-OAc (eq.)	+41!D		*d*
		11β-OH (ax.)	+77D		*d*
		11β-OAc (ax.)	+162D		*d*
12	5β-Cholanate	none	+10		*d*
		11α-OH (eq.)	+17!	+7!	*d*
		11β-OH (ax.)	+39	+29	*d*
12	5β-Etianate	none			
		11α-OAc (eq.)	+30!D		*d*
		11β-OAc (ax.)	+117!D		*d*

[a] Own work.

[b] C. Djerassi, O. Halpern, V. Halpern and R. Riniker, *J. Amer. Chem. Soc.* **80**, 4001 (1958).

[c] C. Djerassi, W. Closson and A. E. Lippman, *J. Amer. Chem. Soc.* **78**, 3163 (1956).

[d] C. Djerassi, *et al.*[15]

[p] Prof. H. B. Henbest, Belfast.

[q] Dr. T. G. Halsall, Oxford.

[r] Prof. E. R. H. Jones, Oxford.

[s] Dr. O. Schindler, Basel.

* Solvent, methanol, except for values marked D which are in dioxan.

† For previous work see chiefly Djerassi *et al.*[15]

§ Compound may have boat conformation of ring A.

SUMMARY FOR AXIAL α-KETOLS

C:O	COH	Octant behaviour*
3 (5α)	2	√
4	5α	√
6	5α	√
11	9	√
11	12	×
12	11	×

* √ Normal × Abnormal

TABLE 4. SUMMARY OF HEXAHYDROINDANONES

Compound	Significant atoms: Cyclopentanone ring	Significant atoms: Adjacent cyclohexanone ring	Significant atoms: Remote rings	Cotton effect: Estimated	Cotton effect: Found (a)	References
			trans-2-*Ketones*			
trans-Hexahydroindan-2-one	2 — —	—	none	— —	−222	*a*
Ditto, with 8-methyl	2 — —	—	none	— —	−219	*b*
16-Oxosteroid	2 — —	—	±	— —	−264	*b*
2-Oxo-5α-Anorsteroid	2 + +	+	+	+ +	+234	*b*
2-Oxo-4,4-dimethyl-5α-A-norsteroid	2 + +	+	+	+ +	+221	*f*§
			trans-1-*Ketones*			
trans-Hexahydroindan-1-one (8-methyl)	1 +	+	none	+	+88	*b*
17-Oxo-14α-steroid	1 +	+	+	+ +	+140	*b*
17-Oxo-18-nor, 14-methylsteroid (from dammarane)	1 +	+	+	+ +	+180†	*d*
15-Oxo-14α-steroid	1 +	+	+ ? N.O.*	+	+120	*c*
Trisnor-17β-hopan-21-one	1 — **	—	±	—	−208	*e*
3-Oxo-5α-A-nor-steroid	1 — **	—	—	— —	−199	*f*
3-Oxo-5α-A-nor-19-nor-steroid	1 —	—	—	— —	−215	*f*
			cis-2-*Ketones*			
2-Oxo-5β-A-norsteroid	2 — —	+	—	—	−150	*b*
cis-Hexahydroindan-1-one (8-methyl) A††	1 —	+	none	—	(+20	*g*
(8-methyl) B	1 +	+	none	+ +	((approx)	
17-Oxo-14β-steroid	1 —	+	±	?	+34	*b*
3-Oxo-5β-A-norsteroid	1 +	+	+	+ +	+133	*b*
Trisnor-17α-hopan-21-one	1 +	+	+	+ +	+118	*e,h*
15-Oxo-14β-steroid	1 —	—	± N.O.*	—	−125	*k*
Trisnorlupan-19-one	1 +	+	± N.O.*	+	+80	*h*
17-Oxo-13α-steroid (18-nor, 14-methyl; from dammarane)	1 —	—	—	—	−110 (approx)	*d*

[a] Bourn and Klyne[21].
[b] Djerassi, Riniker and Riniker[25].
[c] C. Djerassi, W. Closson and A. E. Lippman, *J. Amer. Chem. Soc.* **78**, 3163 (1956).
[d] J. Bielmann, P. Crabbé and G. Ourisson, *Tetrahedron* **3**, 303 (1958).
[e] Own work (unpublished); samples from Dr. T. G. Halsall (Oxford).
[f] G. Ourisson, *et al.*, unpublished.
[g] Acklin and Prelog[22].
[h] C. Djerassi, J. Osiecki and W. Closson, *J. Amer. Chem. Soc.* **81**, 4587 (1959).
[i] R. Hanna, C. Sandris and G. Ourisson, *Bull. Soc. Chim. Fr.* 1454 (1959).
[k] Lardon, Sigg and Reichstein[23].

* Includes atoms in near octants; effects doubtful.
** Also angular methyl group.
† Approximate estimate from incomplete curve.
†† Two conformations, A, steroid-like; B, steroid-unlike.
§ The contributions of the *gem*-dimethyl group at C-4 apparently cancel almost exactly: this illustrates the character of "bisectional" bonds.[19]

analogue (XII, R = H) (cf. Hückel, Linstead), and the R.D. curve of this, measured in our laboratory, shows a large negative Cotton effect almost identical with that of (XII, R = Me).[21] The angle methyl group is not, therefore responsible for the large Cotton effect; inspection of models in the light of the Octant Rule led to the conclusion that it would not be responsible.

The asymmetry of the structure (XII, R = H) lies, then, in the ring system itself and consideration of models leads us to suggest that the asymmetry of the two atoms

[21] P. M. Bourn and W. Klyne, *J. Chem. Soc.* 2044 (1960).

C-8 and C-9 (or of the bonds marked * * in formula XIIA) with reference to the carbonyl group is responsible for the very large Cotton effect.

These tentative ideas were discussed with colleagues in Zürich and Basel, several of whom (notably Professor Prelog) emphasized that this skewed ring system in XIIA contrasts sharply with the symmetrical ring system in a cyclohexanone (XIII), where any asymmetry is of "second-order" due to substituents, not to the ring itself. It is therefore not unreasonable to ascribe the large measure of asymmetry in the carbonyl group of XII to these skewed ring-atoms.

We do not know the *exact* shape of the cyclopentanone ring[16–20] although the latest evidence seems to suggest a 'half-chair' conformation. It is convenient to assume that the shapes of the structures built up from atomic models with the regular valency angles represent approximately the true shape of the molecules. (The author uses the models made by Crystal Structures Ltd., Cambridge.)

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We then obtain for the *trans*-2-ketones of type XII the picture XIIA, in which the *two* atoms C-8 and C-9 and the *two* ring-bonds which are hyperconjugated with the carbonyl group (Cl-8 and C3-9) are skewed in relation to the symmetry planes of this group.

The experimental values for the amplitudes of (XII, R = H) (−222) and (XII, R = Me) (−219) shows that the contribution of these skewed bonds (or the atoms C-8, C-9) to the Cotton effect is of the order of −200; that the effect of the angle methyl group is negligible is explained on the grounds that it is rather near the vertical symmetry plane of the octant system and its 'second-order' effects are overwhelmed by the 'first-order' effects of the ring system.

Extended trans-2-*ketones*. Two important examples of this class are the well-known 16-oxo and 5α-Anor-2-oxosteroids (XIV and XV). In both cases the additional rings are roughly symmetrical about the horizontal plane; in accordance with this the amplitudes (−264, +234) are not greatly increased. Ourisson (ref. *j*, Table 4) has measured the corresponding 2-oxo compound with *gem*-dimethyl at C3 (XVI, R = R′ = H). Any monomethyl analogues (XVI, R = H, R′ = Me or *vice versa*) would be interesting to study.

These results and those for groups subsequently discussed are summarized in Table 4.

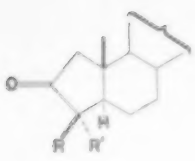
trans-*Hexahydroindan*-1-*ones*. The 8-methyl-*trans*-hexahydroindanone-1 (XVII), which is the bicyclic analogue of a 17-oxosteroid, shows a moderate positive amplitude (+88). The picture shown in XVIIA is compatible with this; a *single* atom in the cyclopentanone ring (C-9) is severely skewed in a positive octant (and C-3 is less skewed in a negative octant). There are also some "ordinary" positive contributions from C-5 and C-6 (C-7 and C-8Me cancel *very* roughly).

The increased amplitude of 17-oxosteroids (XVIII) is due to added atoms (of the A and B rings) in a positive octant (*a*, +140). The ring B-aromatic steroid (3-hydroxy-oestra-5,7,9(10)-trien-17-one) in which the A and B rings are nearer to a symmetry plane (XIX; *a*, +75!) shows an amplitude nearer to that of XVII.

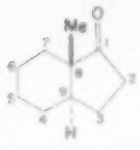
The 14α-15-oxosteroid (XX) shows an amplitude similar to that of the 17-oxo compound (+120 to +140); this case might be complicated by atoms of rings A and B coming in a near octant.

Three other examples from the work of Ourisson are included in Table 4.

cis-*Hexahydroindan*-2-*ones*. Only one example is available here, the 5β-A-nor-2-oxo



XVI

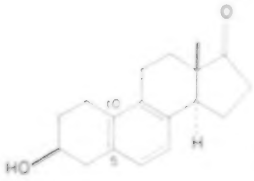


XVII

XVII A

XVIII

17-Oxo-14α-steroid



XIX

XX

15-Oxo-14α-steroid

XXI

2-Oxo-Anor-5β-steroid

steroid XXI (a, −150). Here we have *two* skewed negative ring atoms (C-5 and C-10, two appreciable positive combinations from next nearest atoms C-7 and C-19, and number of very small negative contributions in rings C and D.

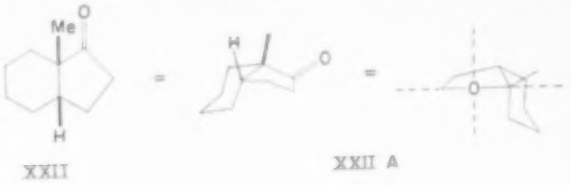
cis-*Hexahydroindan*-1-*ones*. The simple compound XXII has been recently measured (Acklin and Prelog[22]) and found to have a small positive Cotton effect (+20, approx.). The picture shown by XXIIA—the conformation favoured by

[22] W. Acklin and V. Prelog, *Helv. Chim. Acta* **42**, 1239 (1959).

Prelog—does not seem to fit this value well. XXIIA would have a single skewed ring atom (C-9; negative) and two small positive contributions from C-5 and C-6 (C-7 and C-8Me cancel). The alternative conformation XXIIB would have a single *positive* skewed ring atom and some positive contributions from the six-membered ring—i.e. its total Cotton effect would be rather strongly positive.

The disagreement of the experimental result with these predictions suggests that the true conformation must be a distorted form (perhaps of XXIIA)—or conceivably there is a conformational equilibrium (XXIIA + B).

This matter can be tested further by considering some extended compounds of the steroid series. The 14β-17-oxosteroid (XXIII; *a*, +34) has a number of additional atoms (in the B and A rings) in both positive and negative octants; XXIII *must* have



XXII XXII A

or

XXII B

XXIII

17 – Oxo – 14β – steroid

the conformation shown (similar to XXIIA), and the conformation as XXIIB is impossible.

Some other examples are available, in which the keto group is adjacent to a ring junction carrying hydrogen instead of methyl. 3-Oxo-5β-Anor steroids (XXIV) have one skewed positive ring atom, and many ordinary positive contributions; the observed amplitude (+133) supports this analysis. The triterpene derivative, trisnorhopan-21-one (XXV) is a very close analogue of a 3-oxo-5β-Anor steroid; it shows a similar strong positive Cotton effect (*a*, +118).

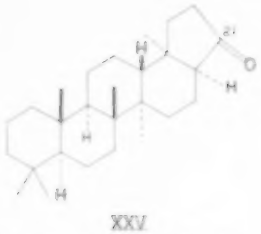
The 15-oxo-14β-steroid (XXVI) (Lardon, Sigg and Reichstein[23]) is roughly enantiomeric to (XXIV) and has (*a*, −125). The picture might be complicated here by 'near octant' effects from rings A and B. 3β-Acetoxy-trisnorlupan-19-one (XXVII) is very similar to the enantiomer of a 15-oxo-14β-steroid; its Cotton effect is positive (+80).

In all the above no account has been taken of the possibility that the six-membered

[23] A. Lardon, H. P. Sigg and T. Reichstein, *Helv. Chim. Acta* **42**, 1457 (1959).

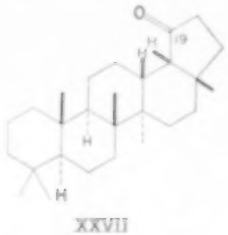
XXIV

3-Oxo-Anor-5β-steroid



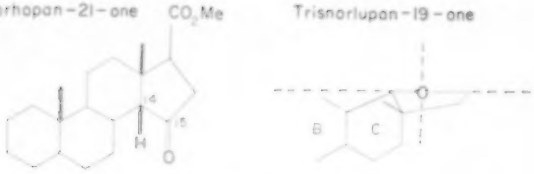
XXV

Trisnorhopan-21-one



XXVII

Trisnorlupan-19-one



XXVI

15-Oxo-14β-steroid

ring adjacent to the cyclopentanone ring may well be a distorted chair, or a form intermediate between a chair and a boat.*

It may be remarked here that the very general and close agreement between the *predictions* of the Octant Rule (based on perfect chair forms of cyclohexane rings) and the *experimental findings* in the extended cyclohexanone series is indirect evidence for the conclusion that the cyclohexane rings are of nearly perfect form in most of the compounds concerned.

Some Bridged-ring Ketones

6. *Bicyclo* [3,2,1] *octan-2-ones*

These compounds (XXVIII–XXX) are only slightly distorted cyclohexanones and a few data collected by Prof. Ourisson suggest that the Octant Rule may be applied to them without difficulty.

(+)-Homoepicamphor (XXVIII) and (−)-homocamphor (XXIX) show positive and negative curves respectively. Measurements were incomplete but the rotations at peak and trough (~310 mμ) respectively indicated that the amplitudes would be approximately ±60. These values tally with the octant diagram which, if one considers the structures as a substituted cyclohexanone, show in each case a (somewhat displaced) 2-axial methyl group having a greater effect than the *gem*-dimethyl at C-3 (cyclohexanone numbering).

"R"-Homocamphenilone (obtained from (+)-camphor) was found by Ourisson to have a strong positive Cotton effect (*a*, +110); this supports the formula (XXX),

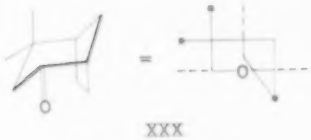
* The results of Djerassi, Marshall and Nakano, *J. Amer. Chem. Soc.* **80**, 4853 (1958) on decalones and related hexahydroindan-5-ones tend to discount this argument.

XXVIII

(+)-Homoepicamphor

XXIX

(−)-Homocamphor



XXX

"R"-Homocamphenilone

XXXI

and not that of the positional isomer (XXXI), which would have a small positive Cotton effect.

7. *Bicyclo* [3,2,1] *octan*-6-*ones*

These are important reference compounds for a number of different groups of natural products. Within the last few months the absolute configurations of two members of the group have been determined by reference to cyclohexanone types obtained by further degradation. They are the nor-ketones (XXXIII) from phyllocladene (XXXII), and (XXXVIII) from gibberellic acid (XXXV). My own attempts to solve the problem of the absolute configurations by direct application of the Octant Rule to these bridged-ring ketones were quite unsuccessful. Perhaps a quantitative treatment may have better fortune.

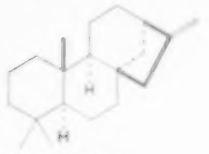
The decalone-type structure (XXXIV) obtained from phyllocladene shows a strong positive Cotton effect (*a*, +81, in dioxan) (Djerassi *et al.*[24]); a similar compound with CN in place of CO_2Me kindly supplied by Dr. R. Hodges of Glasgow gave (*a*, +65!) in our hands. The correlation between phyllocladene and manool achieved by Grant and Hodges[25] fixed the configuration of the A/B ring junction in phyllocladene—and this together with the rotational arguments of Djerassi *et al.*[24] determines the whole

[24] C. Djerassi, M. Cais and L. A. Mitscher, *J. Amer. Chem. Soc.* **81**, 2386 (1959).

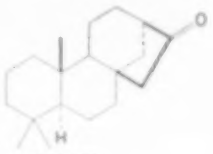
[25] P. K. Grant and R. Hodges, *Tetrahedron Letters* No. 10, 21 (1959) *Tetrahedron* **8**, 261 (1960). R. Henderson and R. Hodges, *Ibid.* **11**, 226 (1960); cf. also L. H. Briggs *et al.*, *Tetrahedron Letters* No. 8, 13 (1959).

stereochemistry of the nor-ketone as XXXIII, and therefore of phyllocladene as XXXII.

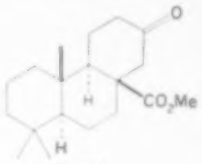
Similar arguments apply to the C/D ring area of the gibberellic acid series, discussed in preliminary notes by Cross, Grove, *et al.*[26] and by Stork and Newman[27].



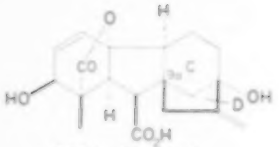
Phyllocladene
XXXII



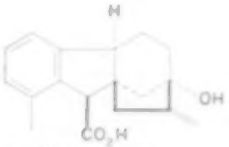
XXXIII



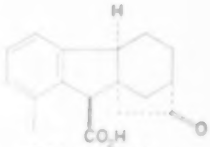
XXXIV



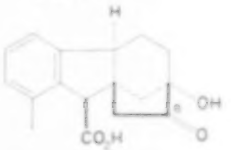
Gibberellic acid
XXXV



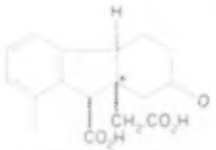
Allogibberic acid
XXXVI



Gibberic acid
XXXVII



Nor-ketone from
allogibberic acid
XXXVIII



Hexahydrofluorenone
type by fission of
ring D
XXXIX

+C.E.
XL

−C.E.
XLI

The hexahydrofluorenones of general type (XXXIX) in which the original configuration at C-9a* is unchanged all have positive Cotton effect curves, indicating the stereochemistry shown. The bridged-ring nor-ketones (XXXVIII) obtained by merely removing the methylene group at C-8 show positive curves; whilst those of type (XXXVII) (gibberic acids) produced by Wagner–Meerwein rearrangement show negative curves.

These results indicate that the bridged-ring bicyclo [3,2,1] structures of general type (XL) have positive Cotton effects, and those of the enantiomeric type (XLI) have negative effects. The application of these reference values (cf. Table 5) to the extensive observations of Djerassi's school on cafestol[24] steviol,[25] and the *Garrya* alkaloids[28] follows logically.

[26] B. E. Cross, J. F. Grove, P. McCloskey, T. P. C. Mulholland and W. Klyne, *Chem. & Ind.* 1345 (1959) and previous references there.
[27] G. Stork and H. Newman, *J. Amer. Chem. Soc.* **81**, 3168, 5519 (1959).
[28] C. Djerassi, B. Riniker and R. Riniker, *J. Amer. Chem. Soc.* **78**, 6362 (1956).

TABLE 5. BICYCLO [3,2,1] OCTAN-6-ONES[a]

	a	Ref.
Type XL		
From phyllocladene	+108 D	28
From gibberellic acid	+52, +88	*a*
From cafestol	+113 D	28
Enantiomeric type XLI		
From steviol	−93	28
Gibberic acid	−90	*a*
Cuachichicine	−19	28

[a] Solvent, methanol, except for values marked D, which are in dioxan.
[a] Own work; cf. also ref.[27]

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8. *Bicyclo* [2,2,1] *heptan-2-ones*

Ourisson and his colleagues[29] have studied a number of these compounds (type XLII) and the following notes represent my own attempt to consider their results in the light of the Octant Rule. This is an alternative treatment to that given by Jacob, Ourisson and Rassat[29] in which they consider the relative *bulk* of two bridges. It is not yet possible to apply the Octant Rule in such a way as to predict the Cotton effect of the fundamental skeleton (XLII), but the contributions of substituents (e.g. methyl groups) generally follow the pattern expected from the Rule. In other words when the substituted compounds are considered in pairs, the differences in amplitude are nearly all of the sign expected. However, the results are not such that one could use them to predict the Cotton effects of any but *very* closely analogous compounds.

All observations and calculations based on models at this stage necessarily assume perfect tetrahedral angles throughout. This assumption is almost certainly incorrect—but at present we have no better data for valency angles etc. available.

In (+)-camphenilone XLIII only two atoms (6 and 7) are significant, since 3a and 3b are in approximately symmetrical positions. The observed amplitude (+18) is therefore the best estimate we have at present for the fundamental skeleton XLII.

The comparison of αα-dimethylcamphor (L) (*a* −50!) and camphor itself (*a* −47) (XLVI) shows that C-3a and C-3b together have a *small* contribution, probably negative. However, since it is small, their contributions do roughly cancel.

The bridge-head methyl group (1a) in fenchone deserves comment. Comparison of (XLV)–(XLIII) indicates that this group has a negligible positive effect (Δa, +3). On the perfect tetrahedral model 1a is just in the upper right octant (*negative*)—but it is so near the vertical C plane (position of which is arbitrary) that any prediction would be unsafe. The *experimental* value alone is significant.

Let us now compare αα-dimethylcamphor (L) and fenchone (XLV). The addition of the *gem*-dimethyl group (7a and 7b) makes a contribution (Δa) of −70 or more. The position of C-7b (very near a vertical symmetry plane) suggests that its contribution would be *small*. C-7a would be expected to have a large negative contribution, but not as big as −70 *a* units. This may be due to repulsion between C-7b and C-3b with consequent distortion of the ring system.

The effect of the *gem*-dimethyl group (C-5a, C-5b) may be estimated by the comparison (XLVII—XLIII) as $\Delta a = -28$. The Octant Rule suggests for C-5a and C-5b moderate negative and small positive contributions respectively; together the contribution should be a small negative one, which agrees with experiment.

Gervais and Rassat, working in Paris,[29a] have noted striking solvent differences for the dispersion curves of isofenchone (XLIX) and epiisofenchone. No explanation of these differences can be given at present, but it is pertinent to draw attention to the findings of Kuhn[30] in classical work on camphor 30 years ago. Kuhn's calculations, taking into account circular dichroism, suggested that the absorption and dispersion curves in the region of 290 mμ might be more complex than at first sight appeared.

To summarize this discussion on bridged-ring ketones; the application of the Octant Rule in this field, as with cyclopentanones, is by no means simple, and many interesting problems remain unsolved. All that has been done here is to set some of the data together.

Plain Curves

It was soon clear from Djerassi's work that plain curves could never give as much structural information as Cotton effect curves; none the less we felt that it was desirable to explore further the field of nonabsorbing compounds (i.e. compounds

XLII

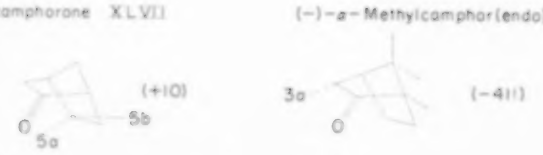
Bicyclo (2,2,1) heptan-2-ones

Reference (29). Values in parentheses are amplitudes; solvent, methanol unless otherwise stated

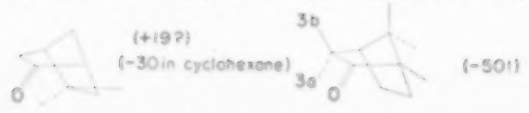
(+)-Camphenilone XLIII (-)-α-Fenchocamphorone XLIV

(+)-Fenchone XLV (-)-Camphor XLVI

(+)-β-Fenchocamphorone XLVII (-)-α-Methylcamphor(endo) XLVIII



(+)-Isofenchone XLIX (-)-$\alpha\alpha$-Dimethylcamphor L



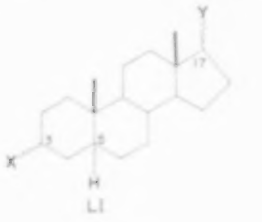
[29] G. Jacob, G. Ourisson and A. Rassat, *Bull. Soc. Chim. Fr.* 1374 (1959).
[29a] H. P. Gervais and A. Rassat, *Bull. Soc. Chim. Fr.* In press (1961).
[30] W. Kuhn and H. K. Gore, *Z. Phys. Chem.* B**12**, 389 (1931); W. Kuhn and H. L. Lehmann, *Z. Elektrochem.* **37**, 549 (1931).

with no absorption above about 280 mμ) since the dispersion curves must necessarily give more information than "monochromatic" rotation measurements at 589 mμ.

Our work has been largely carried out on steroids from our own and colleagues' stocks, and from the Medical Research Council's Steroid Reference Collection, and also on a variety of other natural products.

The questions which we have tried to answer are (i) what are the most suitable data for structural purposes to be obtained from plain curves (Drude constants, dispersion ratios, or simply [ϕ] values at a given wavelength?); (ii) how does vicinal action (between nonabsorbing functions in the same compound) vary with wavelength?; (iii) how closely do the dispersion curves of a homologous series of configurationally related compounds resemble one another?

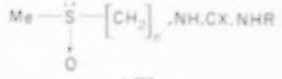
Since the Conference was held, four important papers[30a] on optically active acids have been published by Dr. B. Sjöberg, describing work done in Prof. Djerassi's laboratory.



LI

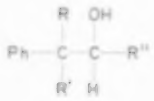
LII

LIII



LIV

Sulphoxides



LV

R = H or alkyl

R', R'' = alkyl

9. *Monosubstituted steroids*

In this work (with Mr. P. M. Jones) we have studied complete series of isomeric 3-, 17- and 20-monosubstituted steroids (LI, LII). The results[31] may be summed up as follows; (i) the rotations—or better the rotation contributions of the substituents (cf. below)—are in all cases much greater at 300 mμ than at 589 mμ; (ii) none of the functions readily calculated from the dispersion curves appears at present to give any

[30a] B. Sjöberg, *Acta Chem. Scand.* (1960); *Ark. Kemi* (1960).
[31] P. M. Jones and W. Klyne, *J. Chem. Soc.* 871 (1960).

more useful *structural* information than the simple value of $[\phi]$ at 300 mμ. These results are very much what might have been expected from the earlier results of Djerassi *et al.*[32*], although a much wider range of functional groups should be studied before any generally valid conclusions can be drawn.

It may be pointed out that while the fundamental steroid hydrocarbon skeleton (5α- or 5β-androstane) has an almost negligible rotation, even at 300 mμ, other steroid hydrocarbons carrying substituents at C-17 (5α- and 5β-pregnanes and cholestanes) have appreciable rotations at 300 mμ (Table 6). These rotations should be subtracted from the rotations of monosubstituted pregnanes and cholestanes to obtained rotation contributions ($\Delta\phi$ values) for the substituents themselves.

TABLE 6. STEROID HYDROCARBONS

	$[\phi]$ at 300 mμ	
	5α	5β
Androstane	+40	−10
Pregnane	+280	+210
Cholestane	+550	+530

10. *Disubstituted steroids*

The complete isomeric series of 5α- and 5β-androstan-3:17-diols and their diacetates and dibenzoates has been studied.[33] In general the vicinal action at 300 mμ follows the same pattern as it does at 589 mμ. In other words for disubstituted compounds where the 'old-fashioned' monochromatic ΔM_D values for the individual substituents are additive, the dispersion curves are additive; for compounds where there is moderate divergence between observed and calculated M_D values, the observed and calculated dispersion curves show a similar moderate divergence.

There is however one apparently glaring exception to the rule of (at least approximate) additivity—viz. 5β-androstane-3α,17α-diol dibenzoate (LIII). The *observed* values for $[\phi]$ were +90 (600 mμ) +170 (500 mμ) and +1460 (300 mμ); the corresponding values *calculated* from those for 3α- and 17α-monosubstituted compounds were −180, −210, −920. No explanation for this behavior can be advanced as yet, but the possibility of "interference" between two benzoate groups on the α-side of the molecule must be envisaged; this might be accentuated if ring A is a deformed chair or boat.

11. *A homologous series—some asymmetric sulphoxides*

No extensive set of rotatory dispersion curves of a homologous series has appeared as such in the recent literature. There are several sets (over restricted wavelength ranges) in the classical literature, and data must be available for a considerable series of aminoacids in the work of the Bethesda group (Greenstein *et al.*[34]), but only Drude constants are given in their papers.

The valuable work of Schellman (see this vol. p. 176 and Chap. 15 of ref. 5) on aminoacids was not available before the Conference.

* Cf. ref. 5, Chap. 1 and 16.

[32] C. Djerassi, E. W. Foltz and A. E. Lippman, *J. Amer. Chem. Soc.* **77**, 4354, 4359, 4364 (1955).
[33] P. M. Jones and W. Klyne, *J. Chem. Soc.* 871 (1960); see also P. M. Jones, M.Sc. Thesis, London (1959).
[34] M. C. Otey, J. P. Greenstein, M. Winitz and S. M. Birnbaum, *J. Amer. Chem. Soc.* **77**, 3112 (1955).

In collaboration with Professor A. Kjaer of Copenhagen[35] we have measured a series of asymmetric sulphoxides of the general type (LIV) where $n = 5$–10, X is S or O, and R is H, Ph or CH_2Ph. As might be expected, the dispersion curves of the various members of this homologous series are almost superimposable. For 12 examples $[\phi]$ (300 mμ) lies between -950 and -1080.

12. *Other work*

Other recent work on plain curves includes studies by Mateos and Cram[36] on aralkyl derivatives of the general type (LV), by Lyle[37] on α-aralkylamines (some of these show partial Cotton effects), by Bose and Struck on diterpenes.[38] We have work in progress on isoquinoline and emetine alkaloids with Dr. A. R. Battersby (Bristol) and on lignans with Professor H. Erdtman (Stockholm).

Acknowledgements—I am greatly indebted to the Wellcome Trust for the generous provision of a spectropolarimeter, to Messrs. Imperial Chemical Industries Ltd. for other equipment, to the Department of Scientific and Industrial Research for a grant and to the U.S. Army Research and Development Group in Frankfurt-am-Main for a contract.

I am grateful to Prof. Carl Djerassi and Prof. Guy Ourisson for the free and continuing exchange of data and ideas; to the many colleagues who have supplied samples for our work, including the pharmaceutical firms who have so generously assisted the Medical Research Council's Steroid Reference Collection; and Mr. P. M. Jones, Mrs. Janet Day and Miss Jane Jackson, who have operated the polarimeter.

[35] W. Klyne, J. Day and A. Kjaer, *Acta Chem. Scand.* **14**, 215 (1960).
[36] J. L. Mateos and D. J. Cram, *J. Amer. Chem. Soc.* **81**, 2756 (1959).
[37] G. Lyle, *J. Org. Chem.* **25**, 1779 (1960).
[38] A. K. Bose and W. A. Struck, *Chem. & Ind.* 1628 (1959).

Tetrahedron, 1961, Vol. 13, pp. 48 to 56. Pergamon Press, Inc. Printed in Northern Ireland

SOME APPLICATIONS OF THE KRONIG–KRAMERS THEOREM TO OPTICAL ACTIVITY

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(*Received* 21 *November* 1960)

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IN general, the speed of a light wave traversing a material medium will be different from its speed in vacuum, and the intensity of the wave will decrease with the distance traveled. The extent to which these phenomena manifest themselves will depend, *inter alia*, upon the frequency ν of the light wave. As is well known, it is a consequence of the geometrical dissymmetry of so-called optically active molecules that the magnitudes of these dispersive and absorptive effects will be different for left and right circularly polarized light in media which contain such molecules. This condition obtains even in homogeneous, isotropic solutions (and our subsequent remarks will be confined to materials of this sort) with the result that such solutions exhibit both circular birefringence and circular dichroism.

Subject to very general assumptions, it may be shown that the dispersive and absorptive effects associated with ordinary (as opposed to rotatory) phenomena are not entirely independent and that from a knowledge of either one of them over the entire region of the spectrum ($\lambda = 0 \rightarrow \lambda = \infty$) one can in fact predict the other. This statement, which will be recognized as a form of the Kronig–Kramers theorem,[1] holds not only for the experimentally observed cumulative absorptions and dispersions, but also for the partial absorption and partial dispersion associated with any particular electronic transition. Physically, of course, this is quite reasonable since both the dispersive and absorptive phenomena take their origins, respectively, in the in-phase and out-of-phase components of the same electronic charge displacements which are induced by the periodic electromagnetic fields associated with the light wave.

The remarks of the preceding paragraph apply also to circular birefringence and circular dichroism with the result that much of the formalism of ordinary and rotatory phenomena is quite similar. This is indicated more explicitly in the outline shown in Table 1.

The important quantity on the rotatory side in Table 1 is R_K, the rotational strength of the K^{th} electronic transition. As has been noted elsewhere,[5] it is R_K that gauges in both sign and magnitude the contribution of an electronic transition to both the rotatory dispersion and the circular dichroism and hence acts as the signed scaled factor that determines how strongly a particular electronic promotion takes part in phenomena associated with optical activity. In addition, as explained below, its value will mirror to a certain extent the nature of the chromophore whose transitions are involved.

Prima facie, one might expect $R_K = \mu_e^K \cdot \mu_m^K$ to be of the order of a Debye times a Bohr magneton, i.e. $\sim 10^{-38}$ cgs. This is indeed the case when the chromophore

[1] R. de L. Kronig, *J. Opt. Soc. Amer.* **12**, 547 (1926); H. A. Kramers, *Atti congr. intern. fisici, Como* **2**, 545 (1927); For a review with extensive references see J. R. Macdonald and M. K. Brachman, *Rev. Mod. Phys.* **28**, 393 (1956).

TABLE 1

Ordinary phenomena	Rotatory phenomena
(1) Partial absorption band associated with the K^{th} transition can be characterized in terms of three parameters (a) wave length of maximum absorption: λ_K (b) a measure of the halfwidth: Δ_K (c) a measure of the area or intensity, the dipole strength: D_K $$D_K = \frac{3hc}{8\pi^3 N_1}\int_0^\infty \frac{k_K(\lambda)}{\lambda}\,d\lambda$$ Here $k_K(\lambda)$ is the partial absorption coefficient for the K^{th} transition. The total mean of absorption coefficient $k = \sum_K k_K(\lambda)$.	(1) Partial dichroism band associated with the K^{th} transition can be characterized in terms of three parameters (a) wave length of maximum ellipticity: $\lambda_K{}^\circ$ (b) a measure of the halfwidth: $\Delta_K{}^\circ$ (c) a measure of the area or intensity, the rotational strength: R_K $$R_K = \frac{3hc}{8\pi^3 N_1}\int_0^\infty \frac{\theta_K(\lambda)}{\lambda}\,d\lambda$$ Here $\theta_K(\lambda)$ is the partial ellipticity associated with the K^{th} transition; N_1 is the number of active molecules per cm^3.
(2) D_K is calculated as the absolute value squared of the electric dipole moment matrix element for the K^{th} transition. We shall abbreviate this as: $$D_K = \mu_e{}^K \cdot \mu_e{}^K$$	(2) R_K is calculated as the imaginary part of the dot product of the electric dipole moment matrix element times the magnetic dipole moment matrix element (exclusive of spin) for the K^{th} transition. We indicate this as: $$R_K = \mu_e{}^K \cdot \mu_m{}^K$$
(3) The real and imaginary parts of the index of refraction or dielectric constant related by the Kronig–Kramers Theorem.	(3) Real and imaginary parts of optical activity (circular birefringence and circular dichroism, respectively) related by a pair of Kronig–Kramers type reciprocal relationships.

itself is inherently dissymmetric (e.g. hexahelicene).[2] However, when the chromophore possesses some higher intrinsic symmetry (e.g. the carbonyl group, which has two orthogonal reflection planes) then most often to a first approximation the transitions of the chromophore will be either electric dipole allowed or magnetic dipole allowed, but not both. It is the latter type of transition (i.e. where $\mu_e{}^K = 0$ to the lowest order of approximation) that lends itself most readily to study by experimental polarimetry. In such instances the optical activity arises through an unsymmetrical perturbation of the chromophoric electrons by a dissymmetric molecular environment in such a way as to provide a small, but nonzero, value for the contribution of $\mu_e{}^K$ to the induced electric dipole moment. In these cases, $\mu_e{}^K$ will be much less than a Debye and the value of R_K will be less than 10^{-38} cgs by perhaps two orders of magnitude or more.

We shall now consider specific calculations involving the two types of chromophores discussed above:

Case I—*The essentially dissymmetric chromophore*

Example. Hexahelicene. When the chromophore is of the inherently dissymmetric type, it can be shown that the shape of the absorption curve and the shape of the dichroism curve must be the same.[3b] Hence, if the rotational strengths can be calculated and if the absorption spectrum be available, one should be able to calculate the

[2] M. S. Newman and D. Lednicer, *J. Amer. Chem. Soc.* **78**, 4765 (1956).

actual shape of the rotatory dispersion curve by means of the reciprocal relations relevant to optical activity. We have performed such a calculation for the molecule hexahelicene,[2] which, as you know, is an aromatic hydrocarbon consisting of six benzenoid rings fused into a roughly helical form.

At the April 1958 ACS meeting in San Francisco we presented our results for the calculation of the rotatory dispersion of hexahelicene in the region from 14,000 to 27,000 cm^{-1}. For that calculation[3a,b] no knowledge of the shape of the dichroism is necessary, for the region considered is sufficiently far removed from any band center

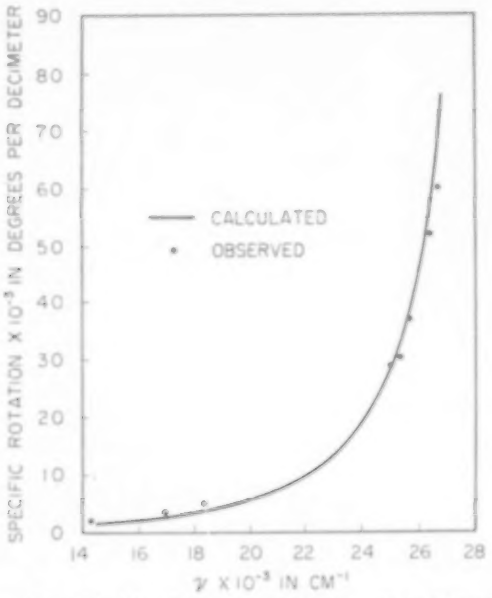

FIG. 1. Rotatory dispersion of hexahelicene (in chloroform)

that each partial rotation is of the Drude form, and the specific rotation [α] may be calculated from the Rosenfeld equation

$$[\alpha] = A \sum_K R_K \frac{\nu^2}{\nu_K^2 - \nu^2} \cdot \frac{n^2 + 2}{3},$$

where A is a known constant, ν_K is the frequency corresponding to the K^{th} transition, and n is the index of refraction of the medium.

Naturally, such a calculation entails certain approximations, and I'll be happy to discuss these during the question period. For the time being, suffice it to say that the wave functions necessary for the computation were obtained from simple Hückel molecular orbital theory and that aside from the need to know the geometry of the molecule and the value of spectroscopic β that enters into Hückel theory, the calculation is a completely *a priori* one. [For another approach to this problem, see D. D. Fitts and J. G. Kirkwood, *J. Am. Chem. Soc.* **77**, 4940 (1955)].

The results are shown in Fig. 1. The agreement between theory and experiment

[3a] A. Moscowitz and J. de Heer, *Division of Physical and Inorganic Chemistry, American Chemical Society Meeting* Paper 25. San Francisco, April (1958). [b] A. Moscowitz, Doctoral Thesis, Harvard University (1957).

was sufficiently encouraging to tempt us to extend the calculation into regions of resonance absorption by the method of using the reciprocal relations, as mentioned above.

For the purposes of the extended calculation, the experimental absorption curve, which was supplied to us by Professor Melvin Newman and Dr. Lini Tsai of Ohio State, was approximated by a sum of three curves Gaussian in frequency corresponding

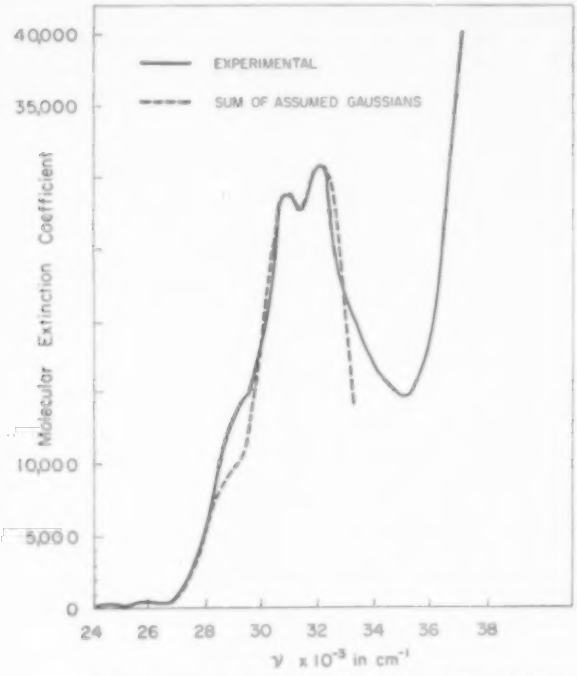

FIG. 2. Absorption spectrum of hexahelicene (in cyclohexane)

to three optically active transitions, as shown in Fig. 2. The results of the computations are shown in Fig. 3 along with the experimental data supplied to us by Drs. Newman and Tsai. (It should be mentioned here that the experimental absorption data were taken with cyclohexane as solvent, but that chloroform is the solvent in the case of the rotation data. This multiplicity of solvents is unfortunate.)

The agreement between theory and experiment is good, especially in view of the approximations that go into the calculation. However, it should be pointed out that because of the large amount of overlapping of the absorption bands under consideration, no unique breakdown into a sum of Gaussians is possible, although the allowed range of possibilities is somewhat limited. As such, the calculation doesn't represent an unequivocal test of the theoretical principles involved. Nevertheless, it is gratifying to note that one can at least achieve a reasonable simultaneous fit to both the absorption and the dichroism where theory predicts that one should exist. And the calculation does provide a further confirmation of the validity of the rotational strengths as computed from simple molecular orbital theory.

It is well to point out here the similarity of our approach to that used by Professor

Kuhn in some of his own calculations. Not only the approach, but the very forms of some of the equations turn out to be quite similar to his, although not identical. This is not mere coincidence, for as Condon[4] has pointed out, the ratio of quantum mechanical quantities, R_K/D_K, which enters into the calculation is closely related to Professor Kuhn's anisotropy factor. Moreover, Professor Kuhn's approach to the

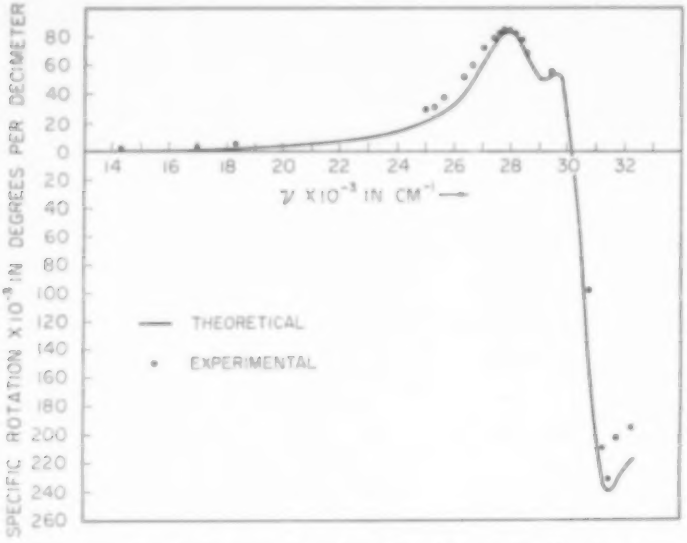

FIG. 3. Rotatory dispersion of hexahelicene (in chloroform)

problem of the relationship between the shape of the partial rotatory dispersion curve and the shape of the associated partial dichroism and absorption curves can be shown to be closely related to the general techniques which employ the integral transform relations if the transitions involved are electric dipole allowed. However, we feel his approach does have restricted validity if such is not the case, as for example in the saturated ketones, which we discuss now.

Case II—*The symmetric chromophore*

Example. The saturated ketones. As noted previously, when the chromophore possesses higher symmetry and is situated in a symmetric molecular environment, as is the carbonyl group in, say, formaldehyde, its associated transitions will be optically inactive. However, if the rest of the molecule be dissymmetrically disposed with respect to such a chromophore (e.g., as in a steroid) then the effect of the environment is to induce a dissymmetry in the electron distribution within the chromophore with the result that its transitions become optically active. In such instances, the rotational strengths for the chromophoric transitions take on an added importance, for they then serve to measure the chromophore's interaction with its dissymmetric molecular environment. Hence, the variation in the rotational strength of a particular transition of a chromophore from molecule to molecule should be fraught with important structural information. And the recent work of Djerassi, Klyne, Schellman and

[4] E. U. Condon, *Rev. Mod. Phys.* **9**, 432 (1937).

others at this symposium has provided ample empirical justification for this point of view.

The first step in the formulation of an organized program to implement these ideas would be to obtain fairly good empirical values for the rotational strengths from the existing dispersion data. The late Professor William Moffitt and I, in close cooperation with Dr. Djerassi, undertook to solve this phase of the problem and we have developed several graphical and computational procedures that will do the job. These

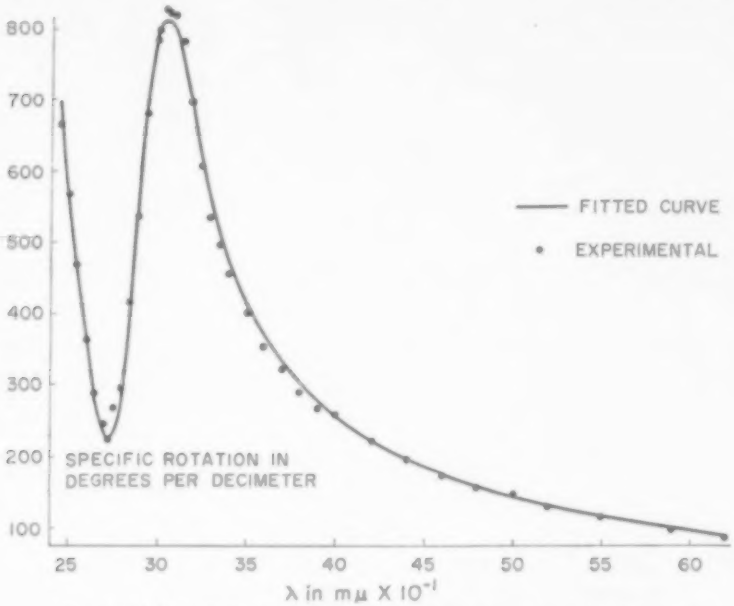

FIG. 4. 12-Ketocholanic acid in methanol [Reproduced from A. Moscowitz, *Rev. Mod. Phys.* **32**, 440 (1960), by permission of the editor.]

entail varying amounts of labor and achieve varying degrees of accuracy. At present we feel that a computational procedure worked out while I was a guest of the Bell Telephone Laboratories and had access to their IBM 704 computer, represents the most satisfactory compromise between tractability and accuracy. Details of the procedure may be found in Dr. Djerassi's book.[5] Just let me say at this point that the method uses the reciprocal relations in conjunction with a least squares curve fitting process for nonlinear functions to pick out, subject to the assumptions inherent in the procedure, the single dichroism curve Gaussian in wave length which best satisfies the data subject to the criterion of least squares. The rotational strength may be determined from the parameters of this curve. Two successful examples of the results of the curve fitting process are shown in Figs. 4 and 5.

It might be well to point out here that in a series of similar compounds the half widths of the best single Gaussians may lie between fairly narrow limits. For example, in most of the saturated ketones we examined, the Δ's for the 290 mμ transition fell between about 18 and 21 mμ. Moreover, in these compounds, the next higher transition is some 90 mμ toward the ultraviolet, so that the background rotation

[5] C. Djerassi, *Optical Rotatory Dispersion* Chap. 12. McGraw-Hill, New York (1960).

contributed by all the transitions other than the one under surveillance will usually have a relatively small curvature in the region between the peak and the trough of the partial dispersion curve associated with the 290 mμ transition. These two facts taken together allow one to show that there will be a rough proportionality between rotational strengths and molecular amplitudes. Advantage was taken of this proportionality in testing the octant rule, already mentioned by Dr. Djerassi in his talk, on a semi-quantitative basis by using molecular amplitudes.

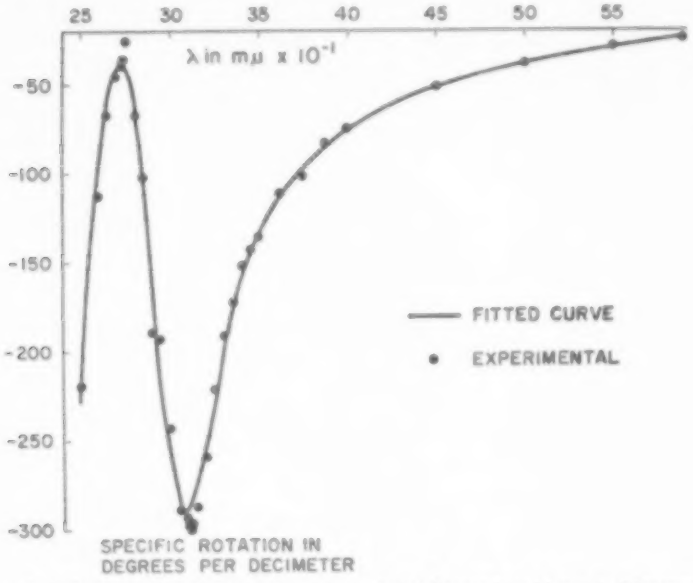

FIG. 5. 3-Acetoxy-cholestan-7-one in methanol [Reproduced from A. Moscowitz, *Rev. Mod. Phys.* **32**, 440 (1960), by permission of the editor.]

Of course, what is ultimately desired is a complete quantitative treatment of the octant rule. And for this we must examine the orbitals involved in the transition. Since the work of McMurry and Mulliken[6], and later Pople and Sidman[7], it has been fairly well established that the 290 mμ transition in saturated ketones involves, roughly speaking, the promotion of an electron from an essentially nonbonding 2p orbital situated on the oxygen atom to an antibonding π orbital involving both the carbon and the oxygen of the carbonyl group. The relevant orbitals are sketched in schematic fashion in Fig. 6.

As Dr. Kauzmann and Dr. Eyring[8] realized twenty years ago, a situation like this is best treated in terms of the one-electron theory. To a first approximation the transition is electric dipole forbidden so that μ_e^1 for the transition will be zero to this order of approximation, although μ_m^1 will be of the order of a Bohr magneton. Here we have set the superscript K = 1 since the transition we are discussing is the first to

[6] H. L. McMurry and R. S. Mulliken, *Proc. Natl. Acad. Sci. Wash.* **26**, 312 (1940); H. L. McMurry, *J. Chem. Phys.* **9**, 231 (1941).
[7a] J. W. Sidman, *J. Chem. Phys.* **27**, 429 (1957); [b] J. A. Pople and J. W. Sidman, *Ibid.* **27**, 1270 (1957); [c] J. W. Sidman, *Chem. Rev.* **58**, 689 (1958).
[8] W. J. Kauzmann, J. E. Walter and H. Eyring, *Chem. Rev.* **26**, 339 (1940).

appear on the long wave length side of the spectrum. The essential point then is to consider in what manner a component of μ_e^1 can be induced in the direction of μ_m^1. This can be brought about in the quantum mechanical formalism by allowing the excited state orbital, which in the coordinate system chosen is formed from a linear combination of $2p_x$ atomic orbitals of carbon and oxygen, to take on some of the characteristics of still higher lying orbitals (e.g. $3d_{yz}$ orbitals) under the action of the dissymmetrically disposed vicinal atoms. The entire excited state molecular orbital so formed will then be neither symmetric nor antisymmetric with respect to the symmetry planes of the carbonyl group and hence $\mu_e^1 \cdot \mu_m^1$ will no longer be zero.

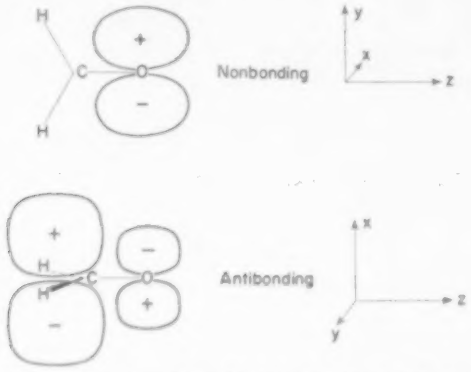

FIG. 6. Diagrammatic representation of the orbitals involved in the 290 mμ carbonyl transition (shown for formaldehyde). [Reproduced by permission from *Optical Rotatory Dispersion* by C. Djerassi. McGraw-Hill, New York (1960).]

A detailed examination shows that if the perturbations produced by the vicinal atoms are spherically symmetric about their own atomic centers, then these perturbations are ineffective if the atoms lie in one of the symmetry planes of the carbonyl group. If the atom does not lie in such a plane, the sign of its perturbation contribution to μ_e^1 will depend upon in which of the four quadrants determined by symmetry planes it lies. A third surface exists which will further divide the quadrants into the eight sign-determining regions which form the basis of the octant rule mentioned by Dr. Djerassi. The exact geometry of this surface will depend upon the relative interactions of the perturbing vicinal atoms with the carbon and the oxygen that comprise the carbonyl group. For the present, this surface is approximated by a third plane perpendicular to the symmetry planes midway along the C=O bond to achieve the eight octants of the present formulation of the octant rule. However, the third surface is most probably not planar in form.

The calculations in which we are presently engaged should shed some more light on the nature of this surface. I had hoped to be able to report on some actual results of the quantitative treatment of the octant rule at this meeting. Unfortunately, at the time I left Minnesota we were still a few weeks away from applying these ideas quantitatively to an actual molecule. All the relevant molecular integrals have been evaluated for the case of perturbing carbon and hydrogen atoms, and it is now just a matter of combining them properly to form the matrix elements pertinent to the geometries of

a series of actual molecules. The perturbation we have invoked is that of the incomplete screening of the nuclei of the vicinal atoms as suggested by the work of Condon *et al.*[9]

As I have already intimated, I think the origins of many of the ideas I have mentioned here can be found in the early work of Condon, Kauzmann and Eyring. At the time at which they worked on the carbonyl problem, the carbonyl orbitals were not sufficiently well characterized nor was there the valuable experimental data available that we have today. The former handicap has been overcome through the work of McMurry and Mulliken and the latter handicap has been removed through the work of Djerassi, Klyne, and others present here today. As such, it may be possible to put the octant rule on a firm quantitative basis. This could lead to a useful set of rules for the structural chemist and would at the same time allow the theoretician to decide which are the important intramolecular forces that give rise to optical activity of the sort just discussed.

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TABLE 2

Compound (in methanol, except where noted)	Exptl. [R_1]	Calc. [R_1]
2,5-dimethyl-cyclohexanone[a]	+1·8	+1·4
2,2,5-trimethyl-cyclohexanone[a]	+6·7	+6·6
trans-9-methyl-1-decalone (dioxane)[b]	+2·7	+3·3
trans-9-methyl-3-decalone (dioxane)[b]	−3·6	−2·1
cis-9-methyl-6-decalone[b]	+0·73	+1·1
1β:9β-dimethyl-*trans*-decalone-3	−0·70	−0·73
cis-10-methyl-1-decalone	−0·74	−0·75
8β,9β-dimethyl-*trans*-decalone-2	+5·1	+5·1

[a] From unpublished data of Djerassi, Osiecki and Eisenbraun
[b] Parameters fixed with these compounds

Note added in proof: Since the time of the meeting, the calculations for a quantitative treatment of the octant rule outlined under Case II in the manuscript have been carried out for a number of saturated ketones in collaboration with Dr. L. C. Snyder of the Bell Telephone Laboratories. The results, which represent work which is only exploratory in nature and which should be considered as only tentative, nevertheless seemed gratifying enough to be worth mentioning here. We have used effective nuclear charges of 1·2 and 1·5 for carbon and oxygen, respectively, in the π-antibonding orbital together with Sidman's coefficients, and we have used an effective charge of unity for both the carbon and oxygen d orbitals. In addition to the effective nuclear charges for the π-orbital, two other parameters were necessary, and these were all determined empirically. Solvent effects were ignored. With these considerations, Dr. Snyder and I find the results indicated in Table 2. The experimental values were obtained from the data of DJERASSI and coworkers by means of the curve fitting procedure already described. The units are those of reduced rotational strength as given in Chapter 12 of Djerassi's book.

[9] E. U. Condon, W. Altar and H. Eyring, *J. Chem. Phys.* **5**, 753 (1937).

Tetrahedron, 1961, Vol. 13, pp. 57 to 105. Pergamon Press, Inc. Printed in Northern Ireland

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THE PRINCIPLE OF PAIRWISE INTERACTIONS AS A BASIS FOR AN EMPIRICAL THEORY OF OPTICAL ROTATORY POWER

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FEW properties of matter can rival optical rotatory power as a potential means of gaining information about the more subtle aspects of molecular structure. Optical rotation is especially sensitive to such structural factors as the spatial conformation of a hydrocarbon chain in an asymmetric molecule and the existence of a six-membered ring in one or the other of the chair forms or bed forms. It is also easy to measure, it is not affected to any unusual extent by impurities, and the apparatus for measuring it is readily available. Furthermore, the theoretical principles that underlie the phenomenon are well understood in a general way. Rosenfeld's quantum mechanical equation for optical rotation specifies the fundamental molecular quantities responsible for the phenomenon and shows that it is dependent on the properties of the electronic transitions that give rise to visible and ultra-violet spectra. The more detailed models of Kuhn, Kirkwood and Condon and Eyring present us with specific ways in which the rotatory properties are related to molecular structure and conformation. These theories have been useful in the interpretation of rotatory dispersion, circular dichroism the role of weak absorption bands, and the effects of solvents and temperature.

Unfortunately the use of optical rotation has not yet reached the level of importance that it deserves. The principal reason for this is that we do not yet have a sufficiently reliable and detailed understanding of the relationship between the optical rotation and molecular structure—an understanding, that is, which would permit us to predict with confidence the sign and magnitude of the rotation to be expected for a given molecule in a given configuration and conformation. All of the existing theories require such drastic approximations when one attempts to use them to calculate a numerical value of the rotation that one can have little confidence in them for this purpose. Furthermore, there is no reason to expect any great improvement in the current theories in this respect in the near future.

At the present time, therefore, the only hope of success in relating optical rotation to molecular structure seems to lie in a more empirical approach. Such an approach has been attempted by a number of workers with varying and uncertain success. One of the earliest attempts was the van't Hoff principle of superposition, which we now know cannot be valid except in certain limited circumstances. More recently Marker[3] Whiffen[4] and Brewster[5] have given discussions of the signs and magnitudes of optical rotations which are encouraging, although the general validity of the principles that they have proposed still remains to be demonstrated.

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[2] Present address: Institute for Molecular Physics, University of Maryland, College Park, Md.
[3] R. E. Marker, *J. Amer. Chem. Soc.* **58**, 976 (1936).
[4] D. H. Whiffen, *Chem. & Ind.* 964 (1956).
[5] J. H. Brewster, *J. Amer. Chem. Soc.* **81**, 5475, 5483, 5493 (1959).

If an empirical approach is to be fruitful, it ought to be set up in a manner consistent with what is known about the general theoretical basis of the phenomenon. In this paper we shall examine these basic requirements. Many theories of optical rotation show that each pair of groups in an optically active molecule can interact to give rise to a contribution to the optical rotation. The contribution by a given pair will in general be affected by the remaining groups in the molecule, but under certain circumstances such disturbances will be small, and it should be a good approximation to express the optical rotation as the sum of contributions arising from all ways of pairing the groups in the molecule (the principle of pair-wise interactions). We shall examine the degree to which this principle may be valid in different types of optically active molecules and we shall show how the principle may be tested further. We shall also show how it may be useful in studying the absolute configurations and conformations of organic molecules.

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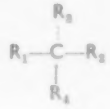
I. ADDITIVITY PRINCIPLES AND THEIR APPLICATION TO OPTICAL ROTATORY POWER

Many empirical theories have been developed which relate the properties of matter to its molecular constitution. Properties which have been successfully dealt with in this way include the molecular volume, the molar energy, the polarizability (and the closely related property of molar refraction), the dipole moment, bond lengths, chemical reactivity (in certain classes of compounds), infrared and ultraviolet absorption spectra, diamagnetic susceptibility and, recently, nuclear magnetic resonance spectra. The empirical theories that are used to predict these properties are invariably based on some form of the principle of additivity: the property in question is given by an aggregate of numbers, each number being characteristic of an atom, or perhaps a group of atoms present in the molecule. (In the case of the dipole moment, vectors rather than numbers must be combined.) At times, however, one encounters substances in which two or more groups interact to give additional effects not observed when either group is present alone. For instance, this is observed in the "exaltation" of the molar refraction of molecules containing conjugated double bonds, or in the increase in the diamagnetic susceptibility of benzene rings as compared with non-cyclic compounds containing three double bonds. Exaltation effects can, however, be included in the additivity principle if appropriate selections are made of the groups of atoms to which group properties are assigned. Thus it is better to consider the benzene ring as a new kind of group rather than as a collection of three double bonds, especially in interpreting the spectra and heats of combustion of compounds containing phenyl groups.

The explanation of the success of these additivity rules is simple: The rules are valid whenever interactions between groups are small. For instance, the contribution of a methyl group to the molar refraction is about the same if the methyl group is adjacent to a carboxyl group as it is when it is adjacent to another methyl group or a phenyl group or a chlorine atom. The displacement of the electrons in a methyl group when it is placed in an electric field (which is what determines the molar refraction) is about the same, no matter what the neighbors of the methyl group might be.

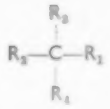
In view of the success of simple additivity rules with other properties, one might be encouraged to construct an empirical theory of optical rotation on the basis of the same type of rule. It is easy to see, however, that (except under certain circumstances which

happen to be found in the sterols; see Klyne[6]) such an approach is theoretically unsound. Consider the asymmetric molecule



(ID)

and its mirror image



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in which R_1, R_2, R_3 and R_4 are simple, optically inactive groups such as —H, —Cl, —CH_3, —COOH, and —C_6H_5. Suppose that we write the optical rotation of compound *ID* as the sum of "partial rotations" assigned to each of its groups, proceeding as though the optical rotation were a property like the heat of combustion:

$$[M]_{ID} = M_1 + M_2 + M_3 + M_4 + M_C$$

where M_1 is the contribution of R_1, M_C is the contribution of the asymmetric carbon atom, and so on. Now let us express the optical rotation of IL by the same procedure. The rotations of ID and IL are equal in magnitude but opposite in sign, so

$$[M]_{IL} = -M_1 - M_2 - M_3 - M_4 - M_C$$

Thus, in some compounds R_1 will have a positive contribution to the rotation, and in others it will have a negative contribution. The decision as to the sign that is to be given to M_1 cannot, however, be based on any property inherent in R_1 because R_1 is assumed to be optically inactive when it stands by itself, so it is not changed when it is replaced by its mirror image. It is clear that the position of each group relative to other groups in the molecule would have to be known in order to be able to give a sign to its contribution. This means that groups in an optically active molecule cannot be considered to be acting independently in contributing to the optical rotation; the contribution by each group is determined by the presence of the other groups. In short no principle of the additivity of independent contributions by groups can serve as a sound basis for a general empirical theory relating structure to optical rotation.

The coupled oscillator theories of Kuhn and Kirkwood and the one electron theory of Eyring and Condon suggest a better procedure for constructing an empirical theory of optical rotation. All of these theories assume that in a molecule such as ID or IL the interactions between the groups, R_1, R_2, R_3 and R_4 affect the motions that the electrons in the groups undergo when the molecule is exposed to light. The optical rotation arises, according to these theories, not from the way in which the electrons move in the isolated groups, but from the way in which these motions are modified because of the presence of other groups in their vicinity. The different theories do not agree in the detailed descriptions of these so-called "vicinal interactions", but this need not concern us. The important point is that all of these theories postulate that the rotation

[6] W. Klyne, *Determination of Organic Structure by Physical Methods*. (Edited by E. A. Braude and F. C. Nachod) p. 108 ff. Academic Press, New York (1955).

comes, not from the sum of contributions by isolated groups, but from the sum of interaction effects between groups.*

This suggests that an empirical theory of optical rotation might be based on the concept of the addition of interactions. As a first approximation we may assume that the important interactions are those that exist between pairs of groups, so that we may write for the optical rotation of compound ID

$$[M]_{1D} = m_{12} + m_{13} + m_{14} + m_{23} + m_{24} + m_{34} + m_{1C} + m_{2C} + m_{3C} + m_{4C} \quad (1)$$

where m_{12} is the optical rotation that would be observed in a hypothetical molecule containing only the two groups R_1 and R_2 located in exactly the same relative spatial positions that they have in compound ID. Similarly m_{1C} is the optical rotation that would be observed if only R_1 and the asymmetric carbon atom were present, and so on for m_{13}, m_{14}, etc. To this approximation the optical rotation of ID is the sum of the pairwise interactions of all of the groups present in the molecule. This assumption will be called *the principle of pairwise interactions*.

Since it has been assumed that group R_3 has some influence on each of the groups R_1 and R_2, it is obvious that the interaction term m_{12} between R_1 and R_2 that would be observed if the group R_3 were not present cannot be quite the same as the interaction between these same two groups when R_3 is present. Similarly, the interaction m_{23} between R_2 and R_3 must be affected by R_1 and the interaction m_{13} between R_1 and R_3 must be affected by R_2. Let us denote by $m_{12.3}$ the change in m_{12} that is caused by the presence of the group R_3. Similarly we may use the symbol $m_{23.1}$ to denote the change in m_{23} that is caused by R_1, and the symbol $m_{13.2}$ to denote the change in m_{13} caused by R_2. Let us next define a "three-way interaction term,"

$$m_{123} = m_{12.3} + m_{23.1} + m_{13.2}$$

Three-way interactions m_{124}, m_{134}, m_{234}, m_{12C}, etc. between other triplets of groups can be defined in a similar fashion. It is evident that if we write

$$[M]_{1D} = m_{12} + m_{13} + m_{14} + m_{1C} + m_{23} + m_{24} + m_{2C} + m_{34} + m_{3C} + m_{4C} + m_{123} + m_{124} + m_{12C} + m_{134} + m_{13C} + m_{14C} + m_{234} + m_{23C} + m_{24C} + m_{34C} \quad (2)$$

* One often sees statements seeming to imply that asymmetric centres in a molecule make contributions to the optical rotation through some unspecified inherent ability to interact with polarized light. The following quotations indicate this: "A full treatment [of the magnitude of the optical rotatory power] should include both asymmetric centers and asymmetric bond arrangements". "In a compound with two or more asymmetric carbon atoms the optical activities of the individual atoms can be added algebraically." Since all physical theories of optical rotation agree that the phenomenon arises exclusively from interactions of one kind or another between groups, it is misleading and fundamentally unsound to base a general empirical approach on any concept of the independent contributions by "centers" to the rotation. (There are, however, special circumstances where this concept may be useful, particularly where two or more asymmetric centers are so far apart in the molecule that the groups about one center do not interact appreciably with the groups about another center. This explains the success of such an approach with the steroids.[5])

It cannot be too strongly emphasized that the role of the asymmetric carbon atom in giving rise to optical rotation is exclusively a structural one. It provides the chromophoric electrons in the molecule with an environment that is not superimposable on its mirror image, and which can be converted into its mirror image only by a rearrangement of chemical bonds—a process requiring a high activation energy and therefore having a low rate. The consequence is that the vicinal actions between groups (taken two at a time if we are concerned with pairwise interactions, or three or four at a time if we wish to consider higher order interactions) fail either to vanish or to cancel one another. The asymmetric carbon atom is, however, only one of a number of ways of introducing a high energy barrier between the enantiomorphs of a compound. (Another way of obtaining such a barrier is through steric hindrance to the rotation of the benzene rings in ortho-substituted diphenyls.) The physical factors that lead to the high energy barrier are entirely different from the physical factors that rotate the plane of polarized light. It is unfortunate that they are so often confused.

we shall obtain a closer approximation to the optical rotation of compound ID than would be given by expression (1). In this approximation the "pairwise interaction terms", m_{12}, etc. are defined in exactly the same way as they are in expression (1) (i.e., m_{12} is still the rotation that would arise if only groups R_1 and R_2 were present). The "three-way interaction term" m_{123} is the correction that would have to be added to $m_{12} + m_{13} + m_{23}$ in order to obtain the optical rotation that would actually be observed if the three groups, R_1, R_2 and R_3, and these three groups alone, were present. The rest of the three-way interactions terms, m_{134}, m_{234}, etc., have similar meanings.

We could, if necessary, go one step further and set up "four-way interactions terms", such as m_{1234}, which would denote the correction that must be added to $m_{123} + m_{134} + m_{124} + m_{234}$ in order to obtain the correct rotation when groups R_1, R_2, R_3 and R_4 (but not the asymmetric carbon) are present. Terms of the type m_{1234}, m_{123C}, etc., when added to expression (2) would give a still better approximation to the actual optical rotation of compound ID. Finally, a "five-way interaction term", m_{1234C}, could be introduced which would take care of all the interactions that were not included in the four-way terms; inclusion of this term would, by definition, have to give the correct value for the optical rotation of ID.

One of the most important problems that faces the current theories of optical rotation is to determine how many of these interaction terms must be included in order to obtain a reasonably good quantitative theory of optical rotation. The empirical approach faces the same problem. In general, the interactions that lead to the pairwise interaction terms, m_{12}, etc., are quite small. The three-way interaction terms, m_{123}, etc., result from the compounding of the effect of one small interaction on another small interaction. This leads us to expect that the three-way terms will be much smaller than the two-way terms. We should like to know, however, just how small they are, and under what conditions one can expect to obtain good results by including only the pairwise terms.

The present paper has two principal objectives. The first objective is to examine the conditions under which the principle of pairwise interactions is valid. We shall do this by an empirical approach, looking for molecules in which the pairwise interactions must vanish, so that all of the rotation must come from three-way and higher interactions. The magnitudes of the rotations observed for such molecules will give us some idea of the errors that might be expected if only pairwise interactions are taken into account. The second objective of this paper is to show that if the principle of pairwise interactions is valid it may be used to predict useful and interesting quantitative relationships between the optical rotations of certain compounds. Unfortunately the data necessary for a rigorous test of most of these predictions are not now at hand, but when they become available they will obviously have an important bearing on the basic question of the adequacy of the pairwise interaction principle—a basic assumption common to most of the current theories of optical rotation.

It should be pointed out that in practice the "interactions" of which we speak here may be rather complex. They will, of course, include the direct vicinal actions that are computed by theories of optical rotation. They may also include other kinds of interactions which influence the optical rotation less directly. For instance, the solvation of a group may be influenced by other near-by groups. (The hydration of a carbonyl group in an aqueous solution of a ketone may be changed if a hydroxyl group is attached to a carbon atom close to the carbonyl.) The vicinal actions between a

given pair of groups (call them A and B) will certainly be affected by the manner in which the two groups are solvated. Suppose that a group C changes the solvation of A or B when it is placed next to one of them. Then the group C may have a significant indirect effect on the contribution of the A—B interaction to the optical rotation, even though the three-way optical interactions of A, B and C are small. In general, therefore, it is desirable to apply and test the principle of pairwise interactions in inert solvents if this is possible. It would be even more desirable if optical rotations could be measured in the vapor, but this is unfortunately difficult to do.

Interactions between a pair of groups will also be significantly changed if a third group is added which can resonate with one member of the pair. Steric interactions between groups may also be responsible for an apparent failure of the principle of pairwise interactions. Thus, in a compound such as 3-ethylcyclopentanone the contribution of the interaction between the ethyl group and the carbonyl group will depend on the spatial position of the ethyl group, especially with respect to rotation of the group about the bond that joins it to the ring. Substitution of a bulky group, such as a phenyl, at position 4 in the ring in a *cis* relationship to the ethyl group would undoubtedly have an influence on the spatial position of the ethyl group. Thus the phenyl group may have a large effect on the contribution to the optical rotation arising from the ethyl-carbonyl interaction, even though the optical interactions of the phenyl group with these two groups happened to be small.

It should be stressed that all of the considerations to be discussed in this paper will apply to optical rotations measured at any wavelength. If we happen to restrict our discussion to measurements made at only one wavelength, this is no sense an inherent limitation of the approach that we are taking.

II. SOME EXPERIMENTAL TESTS OF THE PRINCIPLE OF PAIRWISE INTERACTION

A. *Molecules in which the groups attached to the asymmetric carbon atom have axial symmetry.* Consider the optical rotation of α-bromo-propionitrile, CH_3—CHBr—CN, in terms of the principle of pairwise interactions. According to this principle the molecular rotation should be given by

$$[M] = m_{MeBr} + m_{MeH} + m_{MeCN} + m_{MeC^*} + m_{HBr} + m_{HCN} + m_{HC^*} + m_{BrCN} + m_{BrC^*} + m_{C^*CN}$$

where m_{MeBr} is the contribution to the optical rotation arising from the interaction of the methyl group with the bromine, m_{C^*CN} is the contribution of the interaction between the asymmetric carbon atom and the nitrile group, and so on. It is easy to see that all of these pairwise interactions are identically equal to zero. For instance, m_{MeBr} is defined as the optical rotation of the molecular fragment containing only the methyl group and the bromine atom (Fig. 1) This fragment is superimposable on its mirror image, so it can give rise to no optical rotation. Similarly, all of the other fragments shown in Fig. 1 are superimposable on their mirror images and can have no optical rotation. Therefore the rather large optical rotation that is observed for this substance ($[M]_D = 21°$ for the pure liquid, Berry and Sturtevant[7]) must arise entirely from three-way and higher interactions.

[7] K. L. Berry and J. M. Sturtevant, *J. Amer. Chem. Soc.* **61**, 3583 (1939).

In general *the contribution of pairwise interactions to the optical rotation must vanish for any molecule in which all of the groups attached to the asymmetric carbon atom have axial symmetry about the bond joining them to the asymmetric carbon atom* (Kauzmann and Eyring,[8]). Two other compounds are known having axially symmetric groups of this kind: CH_3—$CH(NH_3^+)$—CN, with $[M]_D = 13°$ in water, (Delepine[9]) and Cl—CHI—SO_3^-, with $[M]_D = 36°$ in water (Read and McMath[10]). The

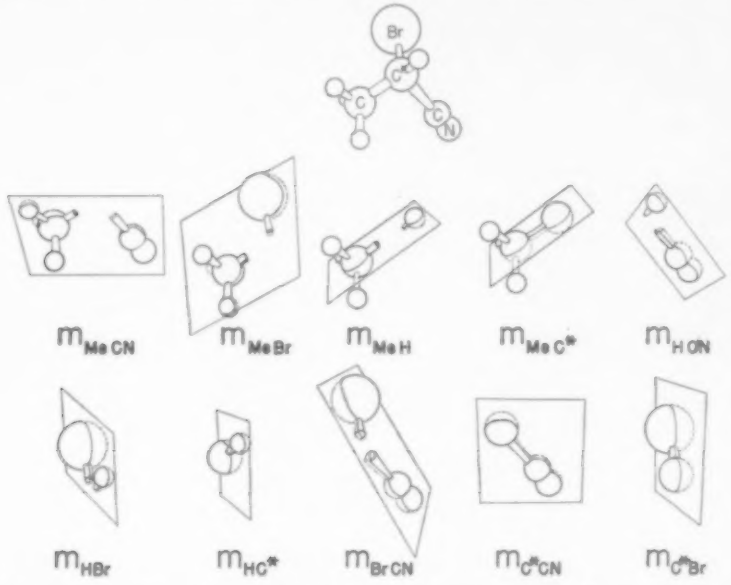


FIG. 1. Cancellation of pairwise interactions in α-bromopropionitrile. If any two groups are considered apart from the rest of the molecule, a plane of symmetry may be passed through them. Therefore pairwise interactions in this molecule cannot give rise to optical rotation.

relatively large rotations of these two substances must also come entirely from three-way and higher interactions.

These examples show that the higher order interactions can lead to optical rotations of sodium D light as large as 20° or 30° or more. It must be realized, however, that in the three examples cited especially large interactions would be expected between the groups attached to the asymmetric carbon atom. At least two of the groups are strongly polar in each of the three examples, so they must have relatively large effects on each other. Furthermore, the interacting groups are all very close together and the measurements were all made in strongly polar solvents, so the indirect effects of groups on each other's solvation may well be pronounced. These rotations must, therefore, be taken as an indication of the upper limit to the magnitudes of three way interactions. The contributions of higher order interactions should be smaller in molecules in which the groups attached to the asymmetric carbon atom are less polar and in compounds in which the polar groups are not so close together; they may also be smaller if the rotations are measured in less polar solvents.

[8] W. Kauzmann and H. Eyring, *J. Chem. Phys.* **9**, 41 (1941).
[9] M. Delepine, *Bull. Soc. Chim. Fr.* (3) **29**, 1195 (1903).
[10] J. Read and A. M. McMath, *J. Chem. Soc.* 2723 (1932).

B. *Molecules in which rotation is possible about the bonds attached to the asymmetric carbon atom.* Consider next the optical rotation of secondary butyl bromide, C_2H_5—CHBr—CH_3. Taking into account the threefold energy barrier that restricts rotation about the carbon-carbon bond joining the ethyl group to the asymmetric carbon atom, this molecule should exist predominantly in the three conformations, A, B and C, shown in Fig. 2. Now let us remove, in our minds, the methyl group and the secondary hydrogen atom from the asymmetric carbon atom, leaving the fragment C_2H_5—C—Br. It will be seen that in forms A and C the conformations assumed by this fragment are mirror images of one another. This means that the contribution to the optical rotation

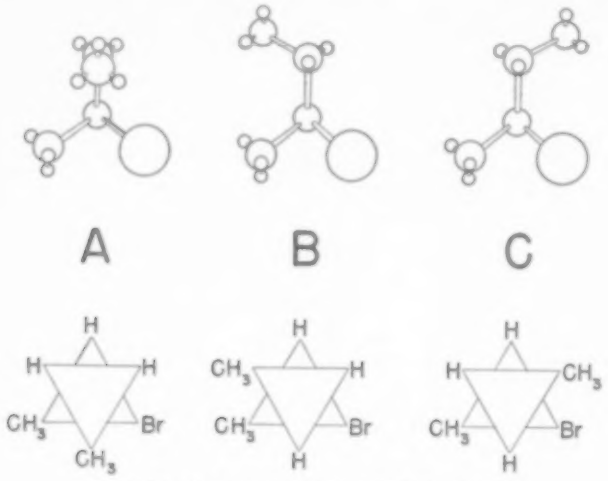


FIG. 2. Conformations of secondary butyl bromide.

arising from the pairwise interaction of the ethyl group and the bromine atom must be equal and opposite in sign in forms A and C. Furthermore, in form B this same fragment is superimposable on its mirror image, so that the contribution of the interaction between the ethyl group and the bromine atom must vanish. The same reasoning will show that the contribution from the pairwise interaction between the ethyl and methyl groups attached to the asymmetric carbon will vanish in form C and will be equal and opposite in sign in forms A and B, and that the contribution of the interaction of the ethyl group and the secondary hydrogen on the asymmetric carbon atom will vanish in A and be equal and opposite in sign in B and C. The contributions of all of the pairwise interactions between the secondary hydrogen atom and the methyl group and the bromine atom vanish in each of the three forms, since the fragments formed from any two of these groups are all superimposable on their mirror images.

It is evident that if secondary butyl bromide existed in equal amounts in the three forms A, B and C, all of the pairwise interactions between its groups would vanish, and the entire rotation would have to arise from higher order interactions. Since higher order interactions generally give smaller optical rotations than pairwise interactions, any forces which cause the three forms to exist in unequal amounts will tend to make the optical rotation larger.

The groups attached to the asymmetric carbon atoms of an optically active molecule will normally be expected to occur in several equilibrium positions which are

symmetrically disposed about the single bond joining the groups to the asymmetric atoms. The arguments presented above are quite general and lead us to expect that *whenever some factor is present which causes these groups to spend unequal amounts of time in the different equilibrium positions, the optical rotation will usually become larger*. We may note by allowing the ethyl group in secondary butyl bromide to occupy its three equilibrium positions to equal extents we have, in effect, given this group an axis of symmetry about the bond that joins it to the asymmetric carbon atom. In a similar fashion it is possible to confer an effective axial symmetry on a propyl or a butyl group (or, indeed, on any group not containing an asymmetric carbon atom that is a member of a ring) by equalizing the populations present in the equilibrium positions about each single bond in the group—including, of course, the single bond between the group and the asymmetric carbon atom. Therefore, this rule is really a corollary of the rule formulated in Section A, above.

The experimental evidence which supports this rule has been presented elsewhere, (Kauzmann and Eyring[8]; Kauzmann, *et al*,[11]) and need not detain us. We shall however, find it instructive to examine the rotations of some compounds in which the groups attached to asymmetric carbon atoms may be expected to attain effective axial symmetry. This will permit us to make some additional estimates of the order of magnitude of the contributions of three-way interactions to the optical rotation in some typical optically active molecules.

(1) *Evidence from the comparison of the rotations of open chain and ring compounds.* The most effective way in which to prevent equalization of the populations in the three positions of equilibrium about the single bonds that are in the vicinity of asymmetric carbon atoms is to make the asymmetric carbon a member of a ring. Organic chemists have known for a long time that ring closure usually brings about a marked increase in the magnitude of the optical rotation, and numerous examples may be cited (Kauzmann and Eyring[8]). Perhaps the most striking examples are to be found in the comparison of the optical rotations of the open chain sugar alcohols with the optical rotations of the cyclitols. Table 1A gives the optical rotations of sugar alcohols of the general formula $H(CHOH)_nH$ and Table 1B gives the rotations of some hydroxylated cyclohexanes. It is seen that the formation of a ring in these chemically quite similar compounds tends to increase the magnitude of the optical rotation by a factor of something like ten.

These two tables force us to the somewhat surprising conclusion that there must be almost complete equalization of the populations in the three equilibrium positions about all of the carbon-carbon bonds in the sugar alcohols of the formula $H(CHOH)_nH$. Table 1B also demonstrates that for the kinds of groups present in these molecules pairwise interactions must be capable of giving optical rotations for sodium D light that may be of the order of 50° to 100°. Finally, Table 1A indicates that the higher order interactions in these compounds must result in optical rotations of less than about 5°. (We cannot say how much less than 5° they are because we do not know how much of of the rotation that is observed for the sugar alcohols is caused by uncompensated pairwise interactions from slight deviations from complete equalization of the populations in the three equilibrium positions about the single bonds. Hydration effects are also undoubtedly large in these compounds because all of the rotations were

[11] W. Kauzmann, J. Walter and H. Eyring, *Chem. Rev.* **26**, 339 (1940).

measured in aqueous solution. These would tend to make the higher order interactions larger than otherwise.)

The conclusion that all conformations of the sugar alcohols are equally likely may come as something of a shock to organic chemists who are concerned with conformational analysis. If one constructs models of these alcohols, and if one follows the usual dictates of conformational analysis in rejecting conformations which tend to bring groups into close proximity, many of the conformations of the open chain sugars

TABLE 1. OPTICAL ROTATIONS OF HYDROXYLATED COMPOUNDS

Substance	Formula	Configurations of hydroxyls*	$[M]_D$ in water†
A. *Fully hydroxylated open chain compounds*			
D-threitol	$HOCH_2(CHOH)_2CH_2OH$	DL	+5·25
D-arabitol	$HOCH_2(CHOH)_3CH_2OH$	DDL	0
D-talitol	$HOCH_2(CHOH)_4CH_2OH$	DLLL	+5·8
D-mannitol	$HOCH_2(CHOH)_4CH_2OH$	DDLL	−3·8
D-sorbitol	$HOCH_2(CHOH)_4CH_2OH$	DDLD	−3·5
D-iditol	$HOCH_2(CHOH)_4CH_2OH$	DLDL	+6·4
D-glycero-L-glucoheptitol	$HOCH_2(CHOH)_5CH_2OH$	DLLDL	+5·1
D-glycero-D-glucoheptitol	$HOCH_2(CHOH)_5CH_2OH$	DDDLD	−1·6
D-glycero-D-idoheptitol	$HOCH_2(CHOH)_5CH_2OH$	DDLDL	+1·5
D-glycero-D-mannoheptitol	$HOCH_2(CHOH)_5CH_2OH$	DDDLL	+4·2
D-glycero-D-altroheptitol	$HOCH_2(CHOH)_5CH_2OH$	DDDDL	−0·6
D-erythro-L-talooctitol	$HOCH_2(CHOH)_6CH_2OH$	DDLDDD	−1·9
D-erythro-L-galaoctitol	$HOCH_2(CHOH)_6CH_2OH$	DDLDDL	+5·8
D-threo-L-galactooctitol	$HOCH_2(CHOH)_6CH_2OH$	DLLDDL	0·0
αzzz-D-gluconitol	$HOCH_2(CHOH)_7CH_2OH$	DDLDDL(L ?)	+4·1
αzzzz-D-glucodecitol	$HOCH_2(CHOH)_8CH_2OH$	DDLDDL(L ?D ?)	+3·6
B. *Hydroxylated cyclohexane derivatives* (cyclitols)			
Viboquercitol	$C_6H_7(OH)_5$	1,2,4/3,5	−90
Protoquercitol	$C_6H_7(OH)_5$	1,4/2,3,5	+42
Epiquercitol	$C_6H_7(OH)_5$	1,2,3,5/4	−9
Inositol	$C_6H_6(OH)_6$	1,2,5/3,4,6	+117

* For the open chain compounds, the chain is written vertically. Starting at the bottom, the hydroxyls that are attached to asymmetric carbon atoms are labeled in succession, D if they lie on the right hand side of the chain and L if they lie on the left hand side. For the cyclohexane derivatives the ring is assumed to be flattened out in the plane of the page and the ring atoms are numbered from 1 to 6 moving clockwise around the ring. The hydroxyl groups that lie above the plane of the paper appear before the slash (/) and those that lie below the plane of the paper appear after the slash.

† Data from Pigman[12] and Angyal and Anderson.[13]

would have to be rejected. Yet the small rotations of these compounds clearly indicate that all of the conformations must be nearly equal in energy. On the other hand Sheppard and Szasz[14] and Scott, McCullough *et al.*[15] have shown that there is a difference of less than 100 cals between the three rotational trans and gauche conformers of 2,3-dimethyl butane (that is, the conformers that result from rotation about

[12] W. W. Pigman, *The Carbohydrates: Chemistry, Biochemistry, Physiology*. Academic Press, New York (1957).
[13] S. J. Angyal and L. Anderson, *Advanc. Carbohyd. Chem.* **14**, 191 (1959).
[14] N. Sheppard and G. J. Szasz, *J. Chem. Phys.* **18**, 145 (1950).
[15] D. W. Scott, J. P. McCullough, K. D. Williamson and G. Waddington, *J. Amer. Chem. Soc.* **73**, 1707 (1951).

the single bond joining carbon atoms 2 and 3). The three conformers of 2,3-dimethyl butane are therefore present in very nearly equal amounts at ordinary temperatures. Since the steric interactions of two methyl groups on adjacent carbon atoms may not be greatly different from the steric interactions of a pair of hydroxyl groups, and since nearly all of the carbon-carbon bonds of the sugar alcohols are disubstituted at either end with carbon and oxygen atoms (and thus resemble the 2,3 bond in 2,3-dimethyl butane) equal energies for the three positions about each bond in the sugar alcohols might not be too unreasonable. It is interesting in this connection to notice that the

TABLE 2. OPTICAL ROTATIONS OF PARTIALLY HYDROXYLATED OPEN CHAIN COMPOUNDS

Substance	Formula	Configurations of hydroxyls*	$[M]_D$ in water†
1,6-di-deoxy D-mannitol	$CH_3(CHOH)_4CH_3$	DDLL	−31.
D-lyxohexanetetrol-1,2,3,4	$CH_3CH_2(CHOH)_3CH_2OH$	DLL	+6·5
2-deoxy D-sorbitol	$HOCH_2CH_2(CHOH)_3CH_2OH$	DLL	+27.
1-deoxy L-sorbitol	$CH_3(CHOH)_4CH_2OH$	DLDD	−7.
D-rhamnitol	$CH_3(CHOH)_4CH_2OH$	DDLL	−20.
2-deoxy D-allitol	$HOCH_2(CHOH)_3CH_2CH_2OH$	DDD	−31·5 (in methanol)
L-idomethylitol	$CH_3(CHOH)_4CH_2OH$	DLDL	+4·3
D-rhodeitol	$CH_3(CHOH)_4CH_2OH$	DDDD	−2·4
D-isorhodeitol	$CH_3(CHOH)_4CH_2OH$	DDLD	−16.
6-deoxy D-talitol	$CH_3(CHOH)_4CH_2OH$	DLLL	−3·8

* Notation same as in Table 1, with right hand carbon atom toward the top of the page.
† Data from Beilstein and Vogel and Georg[16].

optical rotations of alcohols in which some of the CHOH groups of the sugar alcohols are replaced by CH_2 groups often tend to be several times as large as those of the sugar alcohols themselves. (See Table 2.) In these substances a greater proportion of the carbon-carbon bonds are not disubstituted at both ends.

(2) *Evidence from the effect of temperature on the optical rotation of simple open chain compounds.* One of the most effective means of equalizing the populations in the equilibrium positions about carbon-carbon single bonds is to raise the temperature. Unfortunately the effect of temperature on the optical rotation has not been as widely studied as one could wish for this purpose, but we do have the extensive data of Pickard, Kenyon and their co-workers, who made systematic investigations of temperature effects on many aliphatic and aromatic carbinols, their esters and their ethers over a wide range of temperatures (20°C to 150°C or more in many instances). All of these studies were performed on the homogeneous compounds, so that "solvent effects" (here, the effects of the optically active molecules on each other) are a variable factor impossible to separate from intramolecular conformational effects. Nevertheless the studies throw an interesting light on the question of the relative magnitudes of pairwise interactions and higher order interactions, and they also lead to interesting conclusions about the conformations of alkyl chains in simple molecules at room temperature.

Before considering the measurements of Pickard and Kenyon, it is necessary to discuss a correction caused by the variation of the internal field with the temperature, because it produces an indirect effect on the optical rotation that is of no interest here.

[16] H. Vogel and A. Georg, *Tabellen der Zucker und ihrer Derivate*. Springer, Berlin (1931).

All theories of optical rotation show that a general solvent effect exists due to this internal field. If the internal field can be given by the equation derived by Lorentz (and this is generally believed to be a reasonable assumption, especially under the present circumstances) then it is found that the optical rotation of a substance dissolved in a solvent of refractive index n is

$$[M] = \frac{n^2 + 2}{3}[M]^\circ$$

where $[M]^\circ$ is the optical rotation that the substance would have if its molecules were maintained in the same conformations that they have in the solvent, but if they were surrounded by a vacuum (i.e. if the solvent had an index of refraction of unity). Since the refractive index changes with the temperature, the rotation, [M], may therefore depend on the temperature even though the molecular conformations do not change with temperature. (This factor has been discussed by Beckmann and Cohen[17,18], Rule and Chambers,[19] Kauzmann, *et al.*[11] and Schellman.[20]) In order to avoid this extraneous effect, it is better to consider the temperature variation of the quantity

$$[M]^\circ = \frac{3}{n^2 + 2}[M]$$

rather than of [M]. Unfortunately measurements of the index of refraction are not commonly available over a range of temperatures, but if the Lorentz-Lorenz relation is valid,

$$\frac{n^2 - 1}{n^2 + 2}\frac{1}{d} = K$$

where d is the density and K is a constant for any substance or solution, then it is found that

$$\frac{3}{n^2 + 2} = 1 - Kd$$

so that

$$[M]^\circ = [M](1 - Kd)$$

Thus if one knows the index of refraction at one temperature, and if the densities are known as a function of the temperature, it is an easy matter to find K, and from this the temperature dependence of the Lorentz field factor, $3/(n^2 + 2)$. The correction is often not very great, but it is safer to make it, and in most of what follows it has been made. The constant K is usually around 0·3 cm³/gm and between 20° and 150°C the density of the carbinols and their derivatives change, typically, from 0·83 to 0·71. Therefore the Lorentz factor changes from about 0·751 at 20° to 0·787 at 150°—a total variation of about 5 per cent. In many instances this is an appreciable fraction of the total change of the optical rotation in this temperature range.

The variation of $[M]_D^\circ$ with temperature for a number of simple aliphatic carbinols is shown in Fig. 3. In most instances, but not all, the magnitude of $[M]_D^\circ$ decreases with temperature, in accordance with what we should expect if raising the temperature has

[17] C. O. Beckman and K. Cohen, *J. Chem. Phys.* **4**, 784 (1936).
[18] C. O. Beckman and K. Cohen, *J. Chem. Phys.* **6**, 163 (1938).
[19] H. G. Rule and A. R. Chambers, *J. Chem. Soc.* 145 (1937).
[20] J. A. Schellman, *Compt. Rend. Trav. Lab. Carlsberg.* **30**, 363 (1958).

the dual effect of increasing the effective axial symmetry of groups about the bonds that attach them to the asymmetric carbon atom and of decreasing the interactions with the solvent. Where $[M]_D^\circ$ increases with temperature, the increase is slight. In most instances the value of $[M]_D^\circ$ reached at the highest temperatures is under 10°. The principal exception is isopropyl n-decyl carbinol, for which $[M]_D^\circ$ changes from 25·6° at 0°C to 25·3° at 200°C; in this molecule the steric repulsions between the groups attached to the asymmetric carbon atom are greater than in the other compounds shown in Fig. 3.

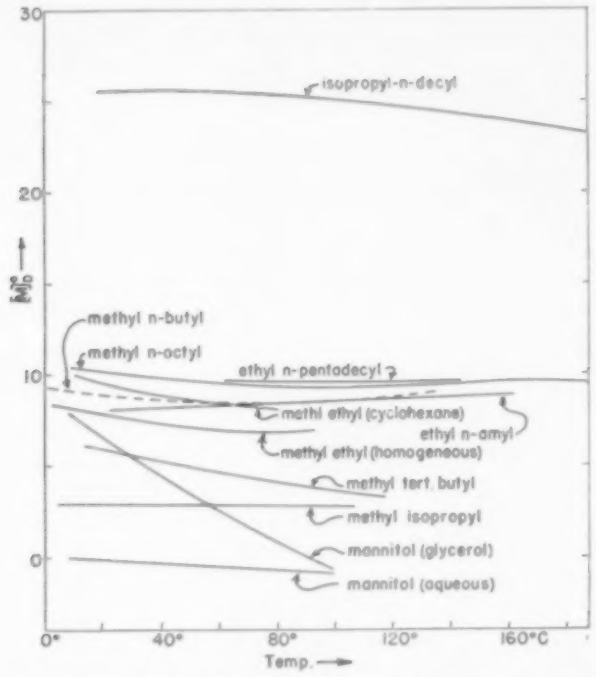


FIG. 3. Temperature dependence of the optical rotations of secondary carbinols (R_1R_2CHOH) and mannitol ($H(CHOH)_6H$). Except where otherwise stated, all rotations were measured on substances in the homogeneous state (no solvent). Data from Pickard and Kenyon (methyl carbinols,[21] isopropyl n-decyl carbinol,[22] ethyl carbinols[23]), Patterson and Todd[24] (mannitol) and Bernstein and Pedersen[25] (methyl ethyl carbinol in cyclohexane).

Several interpretations of these results are possible:

(1) It is possible that in all of these molecules the steric repulsions are so great that even in the temperature range from 0° to 150°C the groups attached to the asymmetric carbon atom are very far from axially symmetric. In this case the observed rotations would be caused principally by pairwise interactions, and the higher order contributions to $[M]_D^\circ$ would be well under 10°. This interpretation seems unlikely because, as we have seen earlier (Section AI), pairwise interactions between groups that are, in an optical sense, not very dissimilar from the alkyl and hydroxyl groups present in these

[21] R. H. Pickard and J. Kenyon, *J. Chem. Soc.* **99**, 45 (1911).
[22] R. H. Pickard and J. Kenyon, *J. Chem. Soc.* **101**, 620 (1912).
[23] R. H. Pickard and J. Kenyon, *J. Chem. Soc.* **103**, 1923 (1913).
[24] T. S. Patterson and A. R. Todd, *J. Chem. Soc.* 2887 (1929).
[25] H. J. Bernstein and E. Pedersen, *J. Chem. Phys.* **17**, 885 (1949).

compounds often result in optical rotations that are considerably greater than 10°. Therefore, if the alkyl carbinols were highly restricted in their internal rotations we should expect to find much larger rotations than we do. We have also seen that in the sugar alcohols, where one would expect the steric repulsions to be at least as great, almost complete axial symmetry probably exists about all of the bonds.

(2) On the other hand it is possible that the steric repulsions in these compounds are sufficiently small that (especially in molecules such as methyl ethyl carbinol, where the groups are small) the groups behave almost as if they were axially symmetric between 20° and 150°C. In this case the higher order contributions to $[M]_D^\circ$ must once again be judged to have an upper limit of about 10°. Temperature-independent values of $[M]_D^\circ$ of 10° or more are observed for methyl butyl carbinol, methyl octyl carbinol, the ethyl alkyl carbinols and isopropyl n-decyl carbinol. All of these compounds have one or more relatively large groups attached to the asymmetric carbon atom. If these groups were to cause a small residual deviation from complete axial symmetry that cannot be removed in this temperature range, then we could conclude that the higher order contributions in compounds of this type must be well under 10°. Unfortunately the $[M]_D^\circ$ values for the smaller carbinols (methyl ethyl carbinol and methyl tertiary butyl carbinol) are available over narrower temperature ranges, because of their lower boiling points, so it is not possible to estimate the limiting value of $[M]_D^\circ$ at high temperatures for them.

Methyl tertiary butyl carbinol is especially interesting because only one of the four groups attached to the asymmetric carbon atom—the hydroxyl group—is not axially symmetric. For this substance $[M]_D^\circ$ shows a greater change with temperature than do most other carbinols. This change may be caused by changes in solvent effects (especially the rupture of intermolecular hydrogen bonds) as well as by changes in molecular conformation (i.e., changes in the orientation of the hydroxyl group about the carbon-oxygen bond). Measurements of the temperature variation of $[M]_D^\circ$ at infinite dilution in an inert solvent would make it possible to separate these two effects. Measurements of this kind have been made by Bernstein and Pedersen[25] on methyl ethyl carbinol dissolved in cyclohexane, and their results are included in Fig. 3. They indicate that the changes in $[M]_D^\circ$ of the homogeneous carbinol observed by Pickard and Kenyon probably arise largely from changes in conformation with temperature, rather than from changes in hydrogen bonding.*

The temperature dependence of the optical rotation of mannitol in water and glycerol has been studied by Patterson and Todd[24] between 15°C and 90°C, and their observations (corrected for the variation in the Lorenz factor) are included in Fig. 3. (Patterson and Todd's observations were made at wavelengths of 579, 546·1 and 435·9 mμ; the rotations for the sodium D line have been estimated by extrapolation of their results, assuming simple Drude-type dispersion). The rotations of mannitol in the two solvents at room temperature are opposite in sign, but on warming the rotations in both solvents move in the levo direction. These results indicate that at high temperatures, where pairwise interactions should vanish, $[M]_D^\circ$ would be more levo than 1°, so we may take this as a lower limit for the contributions of the higher order interactions in

* Bernstein and Pederson have analysed their results assuming that $[M]^\circ$ for methyl ethyl carbinol dissolved in cyclohexane arises entirely from pairwise interactions, and that the contributions of higher order interactions to the optical rotation are so small that they can be neglected. This assumption is highly dubious, so it is not possible to accept their conclusion that the energy of the trans conformations of methyl ethyl carbinol is lower by 800 cals than the energy of the gauche conformation.

this substance. It would be interesting to have similar measurements of the temperature variation of the optical rotations of other sugar alcohols.

The temperature variation of the optical rotations of other types of compounds are shown in Figs. 4–10. The following conclusions may be drawn from these figures.

(1) Conversion of the carbinols to methyl ethers does not appear to affect greatly the magnitude of $[M]_D^\circ$ or its temperature variation (Fig. 4). Ethyl and n-nonyl ethers of methyl n-butyl carbinol have larger values of $[M]_D^\circ$ and the temperature variation

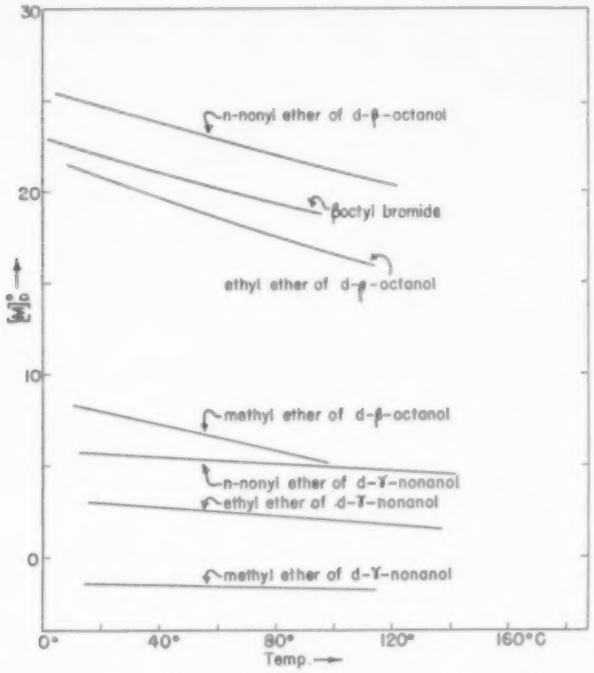


FIG. 4. Temperature dependence of the optical rotations of ethers of secondary carbinols and of β-octyl bromide. Data from Kenyon and McNicol[26] (ethers of d-β-octanol), Kenyon and Barnes[27] (ethers of d-γ-nonanol) and Pickard and Kenyon[21] (β-octyl bromide). Where rotations in the original reference have not been given for the sodium D line, the value has been interpolated from measurements at adjacent wave lengths by means of the Drude formula.

is greater. It appears that in the methyl ethers the effective axial symmetry of the groups attached to the asymmetric carbon atom is about as highly developed as it is in the corresponding alcohols. β-Octyl bromide has a considerably larger $[M]_D^\circ$ than most carbinols, and the temperature variation of $[M]_D^\circ$ is greater. This would indicate that the bulky bromine atom decreases the axial symmetry at room temperature to a greater extent than does the hydroxyl group. Unfortunately it is not possible to estimate the upper limit of $[M]_D^\circ$ at high temperatures for the bromide.

(2) Esters of the type $CH_3—CH(O—CO—R_1)—R_2$, where R_1 and R_2 are normal aliphatic groups (Figs. 5 and 6), have $[M]_D^\circ$ values at high temperatures that are of the order of 10° or less. For the formic acid esters (where $R_1 = H$ with R_2 larger than C_5H_{11}

[26] J. Kenyon and R. A. McNicol, *J. Chem. Soc.* **123**, 14 (1923).
[27] J. Kenyon and T. W. Barnes, *J. Chem. Soc.* **125**, 1395 (1924).

the high temperature values of $[M]_D^\circ$ tend to be somewhat greater, and $[M]_D^\circ$ increases very slightly as the temperature increases. The high temperature values of $[M]_D^\circ$ also tend to be greater when R_1 is a long chain and R_2 is a short chain. Pickard and Kenyon found that the esters show unusually large solvent effects, and that anamolous dispersion is sometimes observed at wavelengths in the visible region of the spectrum.

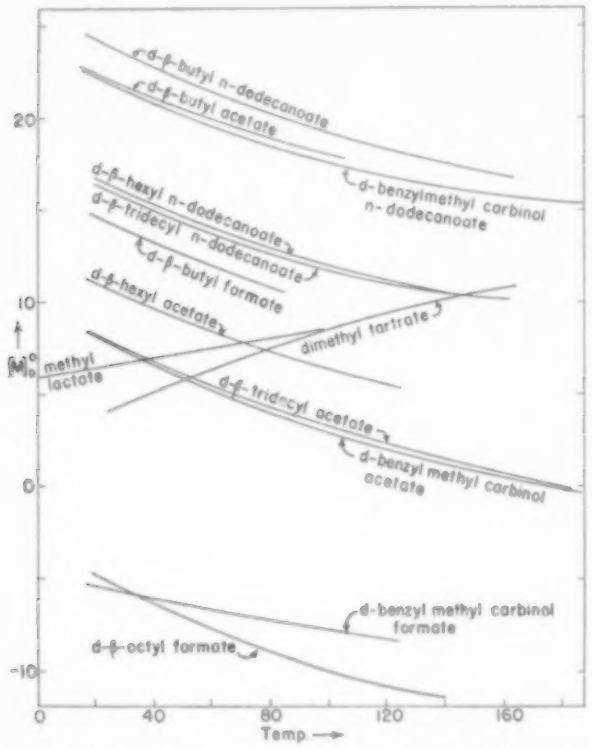


FIG. 5. Temperature dependence of the optical rotations of aliphatic esters of secondary carbinols, CH^2–$CHOH$–R. Data from Pickard and Kenyon[28] (acetates and n-dodecanoates where R is aliphatic), Kenyon and Pickard[29] (benzyl methyl carbinol acetate and n-dodecanoate), Pickard *et al.*[30] (formates), Wood and Nicholas[31] (methyl tartrate), and Patterson and Lawson[32] (methyl lactate). The rotations of methyl lactate were measured at 5790 A. rather than at the sodium D line.

The temperature dependence of the rotation of esters of the type C_2H_5—$CH(C_6H_{13})$—O—CO—R is small (Fig. 6) and $[M]_D^\circ$ values are well under 10° except for R = CH_3 and C_2H_5, where $[M]_D^\circ$ is just under 10°, and for R = H, where $[M]_D^\circ$ is about 20°.

Tartrates and lactates have $[M]_D^\circ$ values which are somewhat greater than 10° at high temperatures. These substances show large solvent effects, and they have two polar groups attached to each asymmetric center, so higher order interactions would

[28] R. H. Pickard and J. Kenyon, *J. Chem. Soc.* **105**, 830 (1914).
[29] J. Kenyon and R. H. Pickard, *J. Chem. Soc.* **105**, 2262 (1914).
[30] R. H. Pickard, J. Kenyon and H. Hunter, *J. Chem. Soc.* **123**, 1 (1923).
[31] C. E. Wood and S. D. Nicholas, *J. Chem. Soc.* 1671 (1928).
[32] T. S. Patterson and A. Lawson, *J. Chem. Soc.* 2047 (1929).

be expected to give larger contributions than in the other compounds being discussed here.

(3) Introduction of aryl groups into the carbinols and their derivatives increases the magnitude of $[M]_D^\circ$, especially if the aryl group is close to an asymmetric center (Figs. 7–10). If the aryl group is attached directly to the asymmetric center (see especially methyl α-naphthyl carbinol, methyl phenyl carbinol and ethyl phenyl carbinol in Fig. 8, and α-naphthyl n-hexyl carbinol and its esters in Fig. 10) the values of $[M]_D^\circ$ at

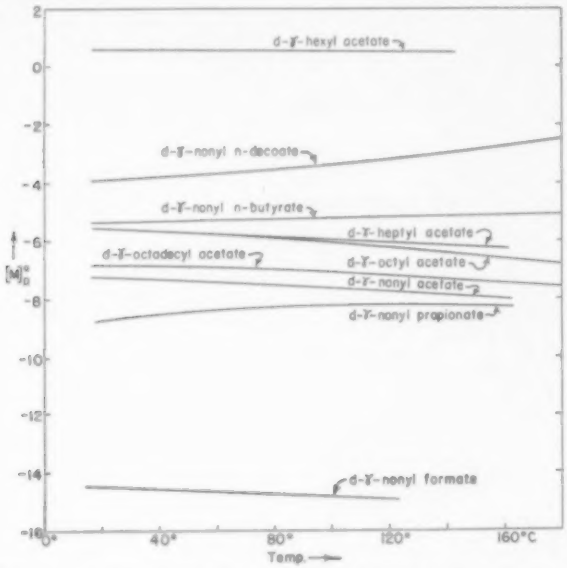


FIG. 6. Temperature dependence of the optical rotations of aliphatic esters of secondary carbinols, C_2H_5-CHOH-R. Data from Pickard, Kenyon and Hunter[30] (formate) and Kenyon[33] (all others).

high temperatures tend to be quite large and the effect of temperature on $[M]_D^\circ$ is also large in most instances. If one or two methylene groups intervene between the aryl group and the asymmetric carbon atom, the magnitude of $[M]_D^\circ$ at high temperatures and the variation of $[M]_D^\circ$ with temperature tend to be smaller. These observations are understandable since the aryl groups are large and rigid and should produce large steric effects that both increase the apparent magnitude of the higher order contributions to the optical rotation (which makes $[M]_D^\circ$ large at high temperatures) and increase the energy difference between the different conformers (which increases the temperature dependence of $[M]_D^\circ$). Furthermore, it is quite likely that the higher order optical interactions that involve aryl groups, alkyl groups and hydroxyl groups or carboxylate groups are larger than those involving a pair of alkyl groups and a hydroxyl or carboxyl.

In summary, we may conclude that in the simple aliphatic carbinols and many of their derivatives at room temperature we have nearly complete three-fold axial symmetry about all of the single bonds. Unfortunately it is not possible to say whether the relatively small optical rotations that are observed for these compounds (of the order of

[33] J. Kenyon, *J. Chem. Soc.* **105**, 2226 (1914).

10°) arise almost entirely from higher order interactions, or whether they are caused by slight deviations from three-fold axial symmetry which could be eliminated by heating to still higher temperatures. In any case, the higher order interactions in these compounds can contribute no more than 10° to the optical rotation (if the molecules are immersed in a medium with refractive index unity), and they may be appreciably smaller. It is highly unlikely that they are completely negligible, however. If more bulky groups are close to the asymmetric carbon atom, and especially if aromatic groups are attached, rotations as large as 50° to 100° may result at high temperatures.

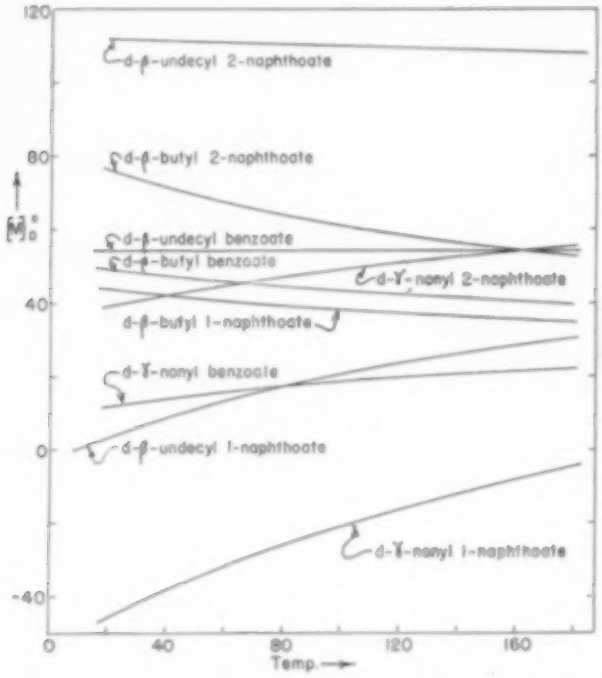


FIG. 7. Temperature dependence of the optical rotations of aromatic esters of secondary aliphatic carbinols. Data from Kenyon and Pickard.[86]

These large rotations may represent true higher order interactions, but they may also include some effects of non-axial symmetry.

(3) *Pairwise interactions and Hudson's rules rules of isorotation.* Hudson showed that if, in a series of sugars, the configuration about one of the carbon atoms is reversed, then the optical rotation is changed in a remarkably regular manner. This is illustrated in Tables 3 and 4. Table 3 shows the changes in rotation that occur when the configuration of the glycosidic carbon atom (C_1) is changed from D- to L- in a number of free sugars and their derivatives. The sugars in this table have been divided into two classes, depending upon the configuration at carbon atom C_2, which is next to the glycosidic carbon atom. In one of the classes (referred to a the "glucose class" because D-glucose is its best known member) C_2 is in the D configuration, and in the

[86] J. Kenyon and R. H. Pickard, *J. Chem. Soc.* **107**, 115 (1915).

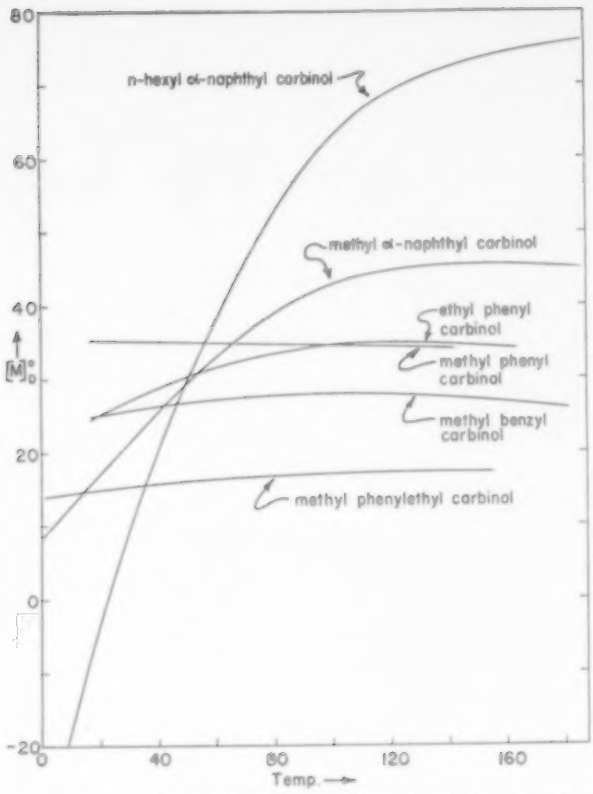
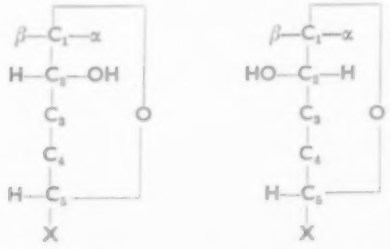


FIG. 8. Temperature dependence of the optical rotations of secondary d-carbinols, R_1–CHOH –R_2, where R_1 is aliphatic and R_2 contains an aromatic group. Data from Kenyon and Pickard[35] (n-hexyl α-naphthyl carbinol) and Pickard and Kenyon[36] (all others).

other class (the "mannose class") the configuration at C_2 is L. In a D-hexopyranose the two classes have the following structures:



where X stands for the group CH_2OH and α and β show the positions of the glycosidic hydroxyl in the α and β forms of the sugar. In the pentoses (xylose, arabinose, lyxose,

[35] J. Kenyon and R. H. Pickard, *J. Chem. Soc.* **105**, 2644 (1914).
[36] R. H. Pickard and J. Kenyon, *J. Chem. Soc.* **105**, 1115 (1914).

ribose), X = H and the α derivative is generally taken as the one in which the configurations at C_1 and C_4 are identical (Bates,[38]). In the heptopyranoses, X = $CH_2OH \cdot CHOH$. In Table 3 these two classes have been further subdivided on the basis of the configurations about the other atoms in the pyranose ring.

Table 3 shows that for free sugars belonging to the glucose class, a change in configuration at C_1 from D to L causes a change in optical rotation of about 175° in most

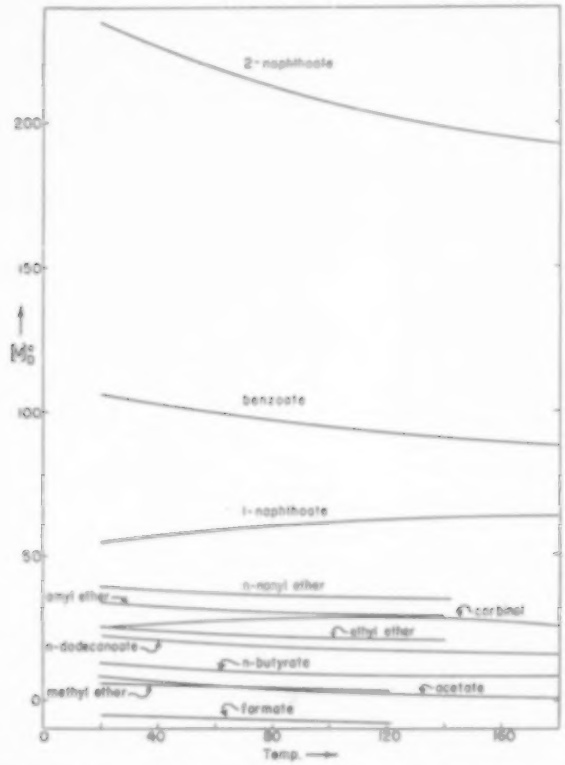


FIG. 9. Temperature dependence of the optical rotations of D-methyl benzyl carbinol and some of its derivatives. Data from Pickard and Kenyon[36] (carbinol), Kenyon and Pickard[23] aliphatic esters other than formate), Kenyon and Pickard[24] (aromatic esters), Pickard *et al.*[30] (formate) and Phillips[37] (ethers).

instances. In the free sugars of the mannose class the same change in configuration results in a rotation difference which is usually considerably smaller, ranging from 75° to 100° in most instances. The rotation changes in free sugars of the mannose class are also somewhat more variable in magnitude than are those of the glucose class. Similar regularities are observed for the rotation differences of each of the other types of derivatives listed in Table 3, although there is somewhat more variation here than for the free sugars.

In general it is found that for a given type of derivative the change in rotation resulting from the inversion of the configuration at C_1 is fairly constant in each class of

[37] H. Phillips, *J. Chem. Soc.* **123**, 29 (1923).

sugar. It is larger and more nearly constant for the glucose class than for the mannose class. The exceptions to this generalization frequently involve the pentose sugars, but exceptions are also found when different solvents were used to measure the rotation of the anomers in an α-β pair, and when one of the members of the pair could not be crystallized (indicating that it might not have been pure).

Table 4 shows that similar regularities are also found when the configurations about

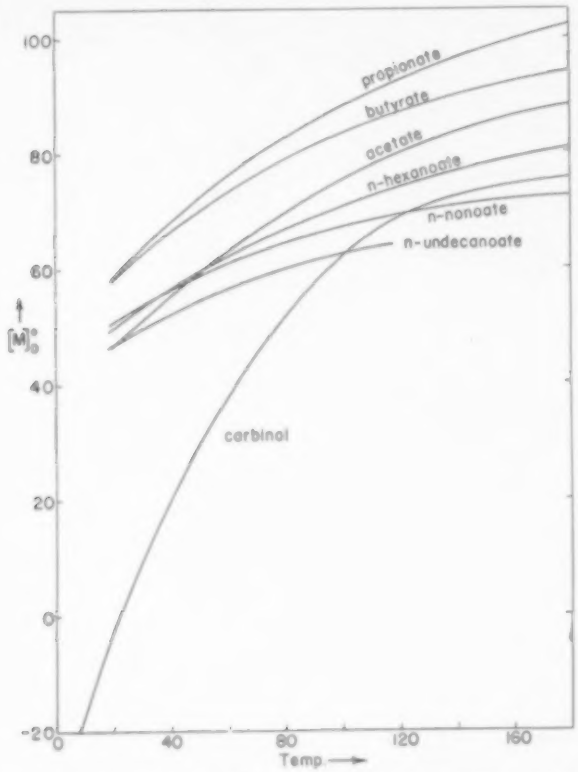


FIG. 10. Temperature dependence of the optical rotation of D-n-hexyl α-naphthyl carbinol and some of its esters. Data from Kenyon and Pickard.[35]

other carbon atoms in the sugar molecule are inverted. The differences in rotation between two substances which differ only in the configuration about one atom are again found to fall into distinct groups, depending (1) on which atom in the molecule is inverted, and (2) on the configurations of the two atoms adjacent to the atom on which the inversion occurs. Table 4A (which is based on a table in Bates[38]) shows this most clearly; here we see the changes in rotation that accompany inversions from L to D at atoms C_2, C_3 and C_4 of the free sugars. If a triad LLD at $C_{n+1}C_nC_{n-1}$ is changed to LDD, the rotation change is about $+150°$ if the atom in the middle of the triad is C_2 and -157 if the middle atom is C_3. If the triad is changed from DLL to DDL, the approximate change in rotation is $-60°$ if the middle atom is C_2, $+80°$ if it is C_3, and $-60°$ if it

[38] F. J. Bates, *Polarimetry, Saccharimetry and the Sugars*. National Bureau of Standards Circular C440, Washington (1942).

TABLE 3. DIFFERENCES IN MOLECULAR ROTATIONS OF α AND β PYRANOSE SUGARS AND THEIR DERIVATIVES
(Hudson's Rule of Isorotation)

Sugar	Free sugar	Methyl glycoside	Phenyl glycoside	Fully acetylated	Acetylated methyl glycoside	Acetylated phenyl glycoside	Fully methylated
A. *Glucose Class* (C_2 in D configuration)							
1. *Ring hydroxyls* DLDD → DLDL*							
D-xylose	—	+360	+538	+363	+524	+655	—
D-glucose	+168	+368	+647	+383	+539	+810	+318†
L-β-galaheptose	—	—	—	+398	+323	—	—
6-methyl D-glucose	—	+322‡	—	+329§	—	—	—
Cellobiose (= 4β-glucosido D-glucose)	—	+413	+768	{+394‖ / +378}	+529	+846	—
Maltose (= 4-α-D-glucosido D-glucose)	—	—	+686	+408	—	+910¶	—
Lactose (= 4-β-D-glucosido D-glucose)	+187	—	—	+396	—	—	—
Gentiobiose (= 6-β-D-glucosido D-glucose)	—	+361	—	+392	+543	—	—
6-deoxy-D-glucose	—	+362**	—	—	+546	—	—
D-glucosamine	—	—	—	+359	—	—	—
2. *Ring hydroxyls* LLDD → LLDL							
L-arabinose	+171	+372	+539	+328	—	—	+421
D-galactose	+176	+380	+669	+319	+533	+856‡	+425‡ ††
D-α-mannoheptose	+177	—	—	+401	—	—	—
D-fucose (= 6-deoxy-D-galactose)	—	+380	—	—	+481	—	—
3. *Ring hydroxyls* LDDD → LDDL							
L-lyxose	+117	+308	—	—	+405	—	—
D-gulose	—	+394	—	—	+469	—	—
D-α-glucoheptose	—	+418	—	+380	—	—	—
4. *Ring hydroxyls* DDDD → DDDL							
D-ribose	—	+341† ‖	—	+347† ‖	—	—	—

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B. *Mannose Class* (C_2 in L configuration)							
1. *Ring hydroxyls* DLLD → DLLL							
D-lyxose	+117	+308	—	—	+405	—	—
D-mannose	+83	+279	+476	{+320‖ / +313}	+360	+581¶	+308
D-α-galaheptose	—	—	—	—	+426	—	—
4-β-D-glucosido-D-mannose	+75	+341	—	+334	+342	—	—
D-rhamnose (= 6-deoxy-D-mannose)	+79	+281	—	—	+303	—	—
2. *Ring hydroxyls* DDLD → DDLL							
D-arabinose	+171	+372	+539	+328	—	—	+421
D-altrose	—	+299	—	—	—	—	—
Celtrobiose (= 4-β-D-glucosido-D-altrose)	—	—	—	+414	—	—	—
Neolactose (= 4-β-D-galactosido-D-altrose)	—	—	—	+411	—	—	—
3. *Ring hydroxyls* LLLD → LLLL							
L-ribose	—	+341†	—	+347†	—	—	—
D-talose	+99	—	—	—	—	—	—
4. *Ring hydroxyls* LDLD → LDLL							
D-idose	—	—	+295‡,‡‡	—	—	—	—

The tabulated figures are the changes in $[M]_D$ resulting from a change in configuration at C_1 from D to L. In the D sugars and their derivatives this means that the rotation of the β form is subtracted from that of the α form; in the L sugars the rotation of the α form is subtracted from that of the β form. Where data for the rotations of a given D or L isomer are not available, the rotation of the enantiomorph has been taken with its sign reversed. Where not otherwise indicated the rotations are taken from Bates[38] for the free sugars, the methyl glycosides, the fully acetylated sugars, and the acetylated methyl glycosides; and from Conchie, *et al.*[39] for the phenyl glycosides and their acetates. Unless otherwise stated, the solvents used are water for the free sugars, the glycosides and the fully methylated sugars; and chloroform for the acetates.

* These letters refer to the configurations of the hydroxyls about atoms C_4, C_3, C_2 and C_1, respectively, with the ring drawn so that the hydroxyl at C_5 would be D. Thus α-D-glucose would be represented by DLDD, β-D-mannose would be DLLL, α-D-arabinose would be DDLD, and α-L-ribose would be LLLL.

† One of the two anomers not obtained in crystalline state; different solvents employed for measurement of rotations of α and β forms.

‡ One of the two anomers not obtained in crystalline state.

§ From Bourne and Peat.[40]

‖ From Conchie, Levvy and Marsh.[39]

¶ Solvent not known for one anomer.

** From Klyne.[5]

†† From Maher.[41]

‡‡ From Bentley.[43]

[39] J. Conchie, G. A. Levvy and C. A. Marsh, *Ad. Carbohyd. Chem.* **12,** 157 (1957).

[40] E. J. Bourne and S. Peat, *Ad. Carbohyd. Chem.* **5,** 145 (1950).

[41] G. G. Maher *Ad. Carbohyd. Chem.* **10,** 257 (1955).

[43] R. Bentley, *J. Amer. Chem. Soc.* **82,** 2811 (1960).

TABLE 4. ROTATION DIFFERENCES RESULTING FROM CHANGES IN CONFIGURATION ABOUT NON-GLYCOSIDIC CARBON ATOMS IN PYRANOSE SUGARS AND THEIR DERIVATIVES

Sugar with OH at C_n in D config.	Sugar with OH at C_n in L config.	Diff. in rotation (D − L)*	Config. of OH at adjacent atoms	
			at C_{n-1}	at C_{n+1}
A. *Free Sugars*				
1. *Pairs of sugars differing in configuration only at* C_2				
α-D-xylose	α-D-lyxose	+132	D	L
α-D-glucose	α-D-mannose	+149	D	L
α-D-galactose	α-D-talose	+149	D	L
α-L-β-guloheptose	α-L-α-gulheptose	+157	D	L
α-D-α-mannoheptose	α-D-β-mannoheptose	+170	D	L
α-D-glucomethylose	α-D-rhamnose	+104	D	L
β-D-allose	β-D-altrose	−59	L	D
β-D-α-glucoheptose	β-D-β-glucoheptose	−60	L	D
β-D-glucose	β-D-mannose	+64	L	L
β-D-galactose	β-D-talose	+71	L	L
α-L-arabinose	α-L-ribose	+85	L	L
β-cellobiose	β-4-β-glucosidomannose	+71	L	L
2. *Pairs of sugars differing in configuration only at* C_3				
β-D-allose	β-D-glucose	−34	D	D
α-D-gulose	α-D-galactose	−157	D	L
β-D-α-glucoheptose	β-D-α-mannoheptose	−157	D	L
β-D-altrose	β-D-mannose	+90	L	D
β-celtrobiose	β-4-β-glucosidomannose	+71	L	D
3. *Pairs of sugars differing in configuration only at* C_4				
α-D-xylose	β-L-arabinose	−146	L	†
β-D-lyxose	β-L-ribose	−140	L	†
α-D-glucose	α-D-galactose	−69	L	D
β-D-glucose	β-D-galactose	−61	L	D
α-D-mannose	α-D-talose	−70	L	D
β-D-mannose	β-D-talose	−54	L	D
α-L-α-galaheptose	α-L-α-guloheptose	−39	L	D
B. *Sugar Derivatives*				
1. *Pairs differing in configuration at* C_2				
a. *Methyl glycosides* (in water)				
Methyl α-D-guloside	Methyl α-D-idoside	+31	D	D
Methyl β-L-arabinoside	Methyl β-L-riboside	+228	D	L
Methyl α-D-xyloside	Methyl α-D-lyxoside	+154	D	L
Methyl α-D-glucoside	Methyl α-D-mannoside	+148	D	L
Methyl α-cellobiose	Methyl α-4-β-glucosido-mannose	+181	D	L
Methyl α-D-gluco-methyloside	Methyl α-D-rhamnoside	+153	D	L
Methyl β-D-guloside	Methyl β-D-idoside	−68	L	D
Methyl β-D-xyloside	Methyl β-D-lyxoside	+102	L	L
Methyl β-D-glucoside	Methyl β-D-mannoside	+68	L	L
Methyl β-D-gluco-methyloside	Methyl β-D-rhamnoside	+72	L	L
b. *Phenyl glycosides* (in water)				
Phenyl α-D-glucoside	Phenyl α-D-mannoside	+171	D	L
Phenyl β-D-glucoside	Phenyl β-D-mannoside	0	L	L

TABLE 4 (*cont'd*)

Sugar with OH at C_n in D config.	Sugar with OH at C_n in L config.	Diff. in rotation (D − L)	Config. of OH at adjacent atoms	
			at C_{n-1}	at C_{n+1}
c. *Polyacetates* (in chloroform)				
β-L-arabinose tetracetate	β-L-ribose tetracetate	+283	D	L
α-D-xylose tetracetate	α-D-lyxose tetracetate	+203	D	L
α-D-glucose pentacetate	α-D-mannose pentacetate	+176	D	L
α-D-galactose pentacetate	α-D-talose pentacetate	+144	D	L
α-cellobiose octacetate	α-4-β-glucosidomannose octacetate	+46	D	L
α-L-arabinose tetracetate	α-L-ribose tetracetate	+308	L	L
β-D-glucose pentacetate	β-D-mannose pentacetate	+113	L	L
β-cellobiose octacetate	β-4-β-glucosidomannose octacetate	−14	L	L
d. *Haloacetates* (in chloroform)				
1-fluo tetracetyl α-D-glucose	1-fluo tetracetyl α-D-mannose	+241	D	L
1-fluo heptacetyl α-cellobiose	1-fluo heptacetyl α-4-β-glucosidomannose	+108	D	L
1-chloro triacetyl β-L-arabinose	1-chloro triacetyl β-L-ribose	+221	D	L
1-chloro tetracetyl α-D-glucose	1-chloro tetracetyl α-D-mannose	+279	D	L
1-chloro heptacetyl α-cellobiose	1-chloro heptacetyl α-4-β-glucosidomannose	+135	D	L
1-bromo triacetyl β-L-arabinose	1-bromo triacetyl β-L-ribose	+250	D	L
1-bromo tetracetyl α-D-glucose	1-bromo tetracetyl α-D-mannose	+272	D	L
1-bromo tetracetyl α-D-galactose	1-bromo tetracetyl α-D-talose	+291	D	L
1-bromo heptacetyl β-cellobiose	1-bromo heptacetyl α-4-β-glucosidomannose	+125	D	L
1-iodo tetracetyl α-D-glucose	1-iodo tetracetyl α-D-mannose	+215	D	L
1-iodo heptacetyl α-cellobiose	1-iodo heptacetyl α-4-β-glucosidomannose	+106	D	L
e. *Methylated sugars* (in water)				
3,4,6 trimethyl α-D-glucose	3,4,6 trimethyl α-D-mannose	+157	D	L
2,3,4,6 tetramethyl α-D-glucose	2,3,4,6 tetramethyl α-D-mannose	+190	D	L
Methyl 2,3,4,6 tetramethyl β-D-glucose	Methyl 2,3,4,6 terramethyl β-D-mannose	+157	L	L
2. *Pairs differing in configuration at* C_3				
a. *Methyl glycosides* (in water)				
Methyl α-D-guloside	Methyl α-D-galactoside	−148	D	L
Methyl β-D-guloside	Methyl β-D-galactoside	−162	D	L
Methyl tetramethyl α-D-altroside	Methyl tetramethyl α-D-mannoside	+137	L	D

TABLE 4 (*cont'd*)

Sugar with OH at C_n in D config.	Sugar with OH at C_n in L config.	Diff. in rotation (D − L)	Config. of OH at adjacent atoms	
			at C_{n-1}	at C_{n+1}
b. *Acetylated methyl glycosides* (in chloroform)				
Methyl tetracetyl α-D-guloside	Methyl tetracetyl α-D-galactoside	−130	D	L
Methyl tetracetyl β-D-guloside	Methyl tetracetyl β-D-galactoside	−66	D	L
3. *Pairs differing in configuration at* C_4				
a. *Methyl glycosides and methylated sugars* (in water)				
Methyl α-D-altroxide	Methyl α-D-idoside	+48	D	D
Methyl α-D-glucoside	Methyl α-D-galactoside	−74	L	D
Methyl β-D-glucoside	Methyl β-D-galactoside	−62	L	D
Methyl 2-methyl β-D-glucoside	Methyl 2M-methyl β-D-galactoside	−75	L	D
2,3,4,6 tetramethyl β-D-glucose	2,3,4,6 tetramethyl β-D-galactose	−118	L	D
Methyl 2,3,4,6 tetramethyl β-D-glucoside	Methyl 2,3,4,6 tetramethyl β-D-galactoside	−94	L	D
3-methyl α-D-glucose	3-methyl α-D-galactose	−89	L	D
b. *Polyacetates* (in chloroform)				
α-D-glucose pentacetate	α-D-galactose pentacetate	−20	L	D
β-D-glucose pentacetate	β-D-galactose pentacetate	−73	L	D
α-D-mannose pentacetate	α-D-talose pentacetate	−52	L	D
α-D-xylose tetracetate	β-L-arabinose tetracetate	−185	L	†
c. *Haloacetates* (in chloroform)				
1-chloro tetracetyl α-D-glucose	1-chloro tetracetyl α-D-galactose	−169	L	D
1-chloro tetracetyl β-D-glucose	1-chloro tetracetyl β-D-galactose	−69	L	D
1-bromo tetracetyl α-D-glucose	1-bromo tetracetyl α-D-galactose	−159	L	D

* Data from Bates[35], Bourne and Peat[40], Bell[43], Bentley[42], Aspinall[44], Hayes and Newth[45], Maher[41,46], Conchie *et al.*[39] and Reeves[47].

† C_3 not asymmetric because sugar is a pentose.

is C_4. If the triad $C_3C_2C_1$ is changed from LLL to LDL then the rotation change is about +70°. Table 4B shows that analogous regularities exist among various types of sugar derivatives, although once again considerably more variation is observed with the derivatives than with the free sugars.

The regularities which Hudson discovered were at first regarded as examples of the van't Hoff superposition principle. It was thought that each asymmetric center in the molecule could be assigned a "partial rotation", and that when the configuration of a

[43] D. J. Bell, *Advanc. Carbohyd. Chem.* **6**, 11 (1951).
[44] G. O. Aspinall, *Advanc. Carbohyd. Chem.* **8**, 217 (1953).
[45] L. J. Hayes and F. H. Newth, *Advanc. Carbohyd. Chem.* **10**, 207 (1955).
[46] G. G. Maher, *Advanc. Carbohyd. Chem.* **10**, 273 (1955).
[47] R. E. Reeves, *J. Amer. Chem. Soc.* **72**, 1499 (1950).

center was inverted the sign of the partial rotation belonging to that center merely had to be reversed. The partial rotation of a center was assumed to be independent of the configurations about the other asymmetric centers in the molecule. It is now known that this cannot be the case, and indeed, the data reported in Tables 3 and 4 are totally inconsistent with such an assumption. Nevertheless, Tables 3 and 4 do demonstrate that numbers can be assigned to each center. These numbers depend on the configurations about adjacent carbon atoms, but they do not depend very much on the configurations about the remaining centers in the molecule. Therefore we may conclude from Hudson's rules that some form of the principle of pairwise interactions must operate in the carbohydrates and their derivatives. The contributions to the optical rotation arising from the interactions between groups located on a pair of adjacent carbon atoms often do not seem to be strongly influenced by the configurations of the groups elsewhere in the molecule.

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(4) *Pairwise interactions and current empirical rules of optical rotation.* Whiffen[4], and more recently Brewster[5], have proposed interesting empirical rules by means of which they have been able to account for the signs, and in many instances even the magnitudes of the optical rotations of a large number of optically active compounds. Both workers have made extensive use of assumptions which are equivalent to the principle of pairwise interactions. Whiffen's approach is based upon the idea that bonds located on adjacent atoms, but arranged relative to one another in a skewed sense, will make a contribution to the optical rotation which depends on the nature of the bonds and the angle between the bonds. The optical rotation of a molecule is merely the sum of these contributions. When a given arrangement of a pair of bonds is replaced by its mirror image, the contribution of this pair is reversed in sign but not altered in magnitude. Brewster distinguishes between "atomic asymmetry", "conformational asymmetry", and "permolecular asymmetry"; the contributions of "conformational asymmetry" to the optical rotation of a molecule are dealt with in a manner very similar to that used by Whiffen, except that more explicit rules are given for computing the magnitudes of the contributions by given pairs of groups.

Both Whiffen and Brewster have included in their treatments only interactions between groups located on adjacent carbon atoms, arguing that the interactions giving rise to optical rotation decrease rapidly with distance between the interacting groups. It is, however, well known that in certain molecular conformations groups attached to non-adjacent carbon atoms may come very close to each other. For instance, two axial groups at positions 1 and 3 of a chair form of a cyclohexane ring are somewhat closer to one another than are the hydroxyls at positions 1 and 2 in any chair conformation. Chair conformations which result in more than one axial group on the same side of the ring are said to be unstable, but there may be instances in which they must occur. On the other hand, as we shall find by means of elementary symmetry arguments, pairwise interactions between symmetrical axial groups (such as chloride, methyl or freely rotating hydroxyl groups) on a chair form of a cyclohexane ring cannot contribute to the optical rotation, so in this particular instance the interactions between groups on non-adjacent atoms may be small. Yet it is always possible that, for instance, a pair of axial hydroxyls at C_1 and C_3 of a cyclohexane ring will not be able to take on an effective three-fold symmetry about the C—O bond, and that their interaction might therefore make a considerable contribution to the optical rotation. Furthermore, it is quite unlikely that the interactions between, say, an equatorial

group at C_1 and an axial group at C_3 of a cyclohexane ring will give a completely negligible contribution to the optical rotation. Therefore the neglect of interactions between non-adjacent atoms must be made with some caution, and a theory will be more accurate if these interactions are included. We shall see that it is frequently not difficult to do this and thus arrive at a somewhat more reliable set of predictions for the optical rotations of certain types of compounds than would be possible using the theories of Brewster and Whiffen.

Other empirical rules have been proposed which are equivalent to the assumption that optical rotation arises exclusively from four-way interactions between the groups attached to an asymmetric atom. This is true of the rules proposed by Crum-Brown and Guye (see Partington[48]) and by Marker.[3] It is also the case for the "atomic asymmetry" contribution discussed by Brewster.[5] Such rules would be most likely to be valid under conditions in which pairwise interactions vanish—that is, at high temperatures in compounds containing asymmetric carbon atoms which are not located in rings, and in compounds in which the groups attached to the asymmetric atoms are not bulky and do not interact strongly with one another by means of hydrogen bonds or other secondary forces. The theoretical approaches of Oseen,[49] Born,[50] de Malleman[51] and Boys[52] would also seem to be most appropriate under these conditions. Brewster has, indeed, shown that in a group of simple compounds which may fulfill the above conditions the sign of the rotation may be predicted successfully from the contribution of the atomic asymmetry alone. It should be pointed out, however, that four-way interactions would be expected to give smaller contributions to the optical rotation than three-way interactions. It would therefore seem more reasonable to start with the concept of three-way interactions in setting up an empirical theory of optical rotation that is to be applied to this type of compound.

III. SOME SIMPLE QUANTITATIVE RELATIONSHIPS BASED ON THE PRINCIPLE OF PAIRWISE INTERACTIONS

In this section we shall show that if the principle of pairwise interactions is valid, interesting relationships should exist between the optical rotations in certain groups of compounds. Many of the compounds that will be discussed have not been synthesized and may not be synthesized in the near future. They are considered as convenient examples to illustrate how the principle may be employed to correlate the optical rotations of configurationally related compounds. It will be evident that the principle may be useful in determining the absolute configurations and conformations of molecules, once we understand the limitations on its validity. The examples should make the application of the principle clear enough to enable the organic chemist to find other, and perhaps more interesting applications. Indeed, as has been mentioned, Whiffen and Brewster have already made extensive use of the principle in a restricted form. Our aim is to show that these restrictions are not necessary in many instances.

It should be emphasized, too, that the establishment of the limits of the validity of the principle of pairwise interactions is of paramount importance to the theoretical

[48] J. R. Partington, *An Advanced Treatise on Physical Chemistry* Vol. 4, p. 341. Longmans, Green, London (1953).
[49] C. W. Oseen, *Ann. Physik.* **48**, 1 (1915).
[50] M. Born, *Physik. Z.* **16**, 251 (1915).
[51] R. de Mallemann, *C.R. Acad. Sci., Paris.* **181**, 298 (1925).
[52] S. F. Boys, *Proc. Roy. Soc.* A **144**, 655, 675 (1934).

understanding of optical rotatory power. We need to know more about the effects of solvents on pairwise interactions, the extent to which solvent molecules participate in pairwise interactions, the influence of steric effects on pairwise interactions, and the relative magnitudes of pairwise and higher order interactions. At the moment this problem is at least as significant as the possibility that the principle may provide a useful tool for the study of molecular configurations and conformations in optically active substances. If progress is to be made with this aspect of the problem we cannot afford to be satisfied with the more limited assumptions of Whiffen and Brewster.

The application of the principle is illustrated in a particularly simple fashion by the three possible optically active methyl cyclopropane derivatives, *trans*-1,2-dimethyl

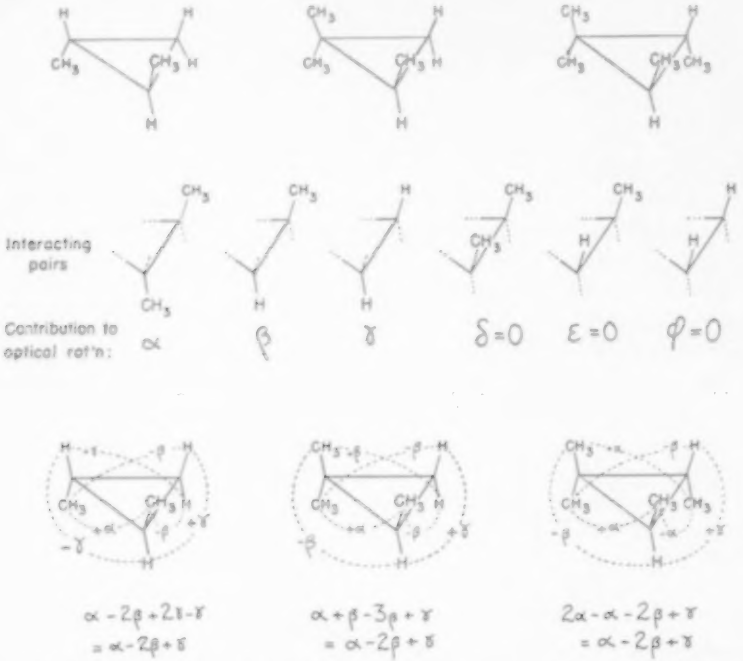


FIG. 11. Application of the principle of pairwise interactions to the three optically active methyl derivatives of cyclopropane.

cyclopropane, 1,1,2-trimethyl cyclopropane and *trans*-1,1,2,3-tetramethyl cyclopropane (see Fig. 11). It will be assumed that the bonds from the cyclopropane ring to the hydrogen atoms and methyl groups are symmetrically disposed on either side of the plane of the ring.*

Six types of pairwise interactions will occur between the groups attached to the ring (the interactions between the three carbon atoms in the ring could not, of course, give

* Since eclipsed configurations about carbon–carbon single bonds are unfavourable energetically, it is possible that this assumption is incorrect. The methylene groups in the cyclopropane ring would then spend much of the time rotated slightly to either side of the plane of the carbon atoms in the ring, and the bonds from the ring would not, at any given moment, be symmetrically disposed on either side of the plane of the ring. Nevertheless, each methylene group would be able to assume at least two equilibrium positions, and on averaging over these multiple positions the six bonds of attachment to the ring would become equivalent. Provided that the equilibrium positions were equally populated, the assumptions made here would then still be completely valid.

rise to any optical rotation because this triplet of atoms is superimposable on its mirror image). These are the interactions that occur between a pair of *trans* methyl groups (making a contribution α to the optical rotation), a *trans* methyl-hydrogen (contribution β), a *trans* hydrogen-hydrogen (contribution γ), a *cis* methyl-methyl (δ), a *cis* methyl-hydrogen (ε) and a *cis* hydrogen-hydrogen (φ). Since the axes of the groups in the three *cis* arrangements are coplanar, however, all of the *cis* pairs of groups are superimposable on their mirror images, so their interactions can give no contribution to the optical rotation, and $\delta = \varepsilon = \varphi = 0$. When the non-vanishing interactions in the three molecules are added together, assuming the absolute configuration shown in Fig. 11, the remarkable result is obtained that all three molecules should have the same rotation, $\alpha - 2\beta + \gamma$. The same result will be true if instead of methyl groups any other axially symmetric groups are present on the ring. Unfortunately such compounds appear to be difficult to prepare in optically pure form, so this result is at the moment of little more than academic interest as a test of the principle of pairwise interactions.

A somewhat similar situation is found for the nine non-enantiomorphic optically active polymethylcyclobutanes (Fig. 12). In this case there is evidence that the ring is not planar (Lemaire and Livingston[53], Dunitz and Schomaker[54], Owen and Hoard[55]) but it is probably safe to assume that the ring undergoes oscillations which make the bonds from the ring in effect symmetrically disposed on either side of the average plane of the ring. (It is difficult to agree with Brewster's contention that in the puckered cyclobutane rings the interferences across the ring would be sufficiently great to eliminate some of the conformations.) Pairwise interactions between methyl groups or hydrogen atoms attached to carbon atoms that lie across the ring from each other will vanish because these atoms and groups are coplanar. Consequently, interactions between groups attached to adjacent atoms on the ring are the only ones which can give rise to optical rotation. As was the case for the methyl cyclopropanes, only interactions between groups in the *trans* relationship to each other need to be considered. Three types of interactions are therefore present in these molecules: The *trans* methyl-methyl, the *trans* methyl-hydrogen, and the *trans* hydrogen-hydrogen. The contributions of these interactions to the rotation are again denoted by α, β and γ, respectively. (The values of these constants will presumably be slightly different in the cyclobutanes and in the cyclopropanes because the spatial relationships are not quite the same.) It is seen from Fig. 12 that seven of these optically active compounds should have the same optical rotation, the eighth should have double the rotation of the rest, and the ninth should have zero optical rotation (despite the fact that it is not superimposable on its mirror image). Of course, the same relationships should hold among any set of derivatives of cyclobutane involving axially symmetric groups.

The thirty-five non-enantiomorphic optically active polymethyl derivatives of cyclopentane provide us with a slightly more complicated example which may also be dealt with by means of the principle of pairwise interactions. The cyclopentane ring is known to be non-planar, but it is also known that the distortions away from planarity are highly mobile and that in cyclopentane itself there exists a plane such that, if a time exposure is made of a given molecule, any one atom in the molecule will spend an equal

[53] H. P. Lemaire and R. L. Livingston, *J. Chem. Phys.* **18**, 569 (1950).
[54] J. D. Dunitz and V. Schomaker, *J. Chem. Phys.* **20**, 1703 (1954).
[55] T. B. Owen and J. L. Hoard, *Acta Cryst.* **4**, 172 (1951).

amount of time in equivalent positions on either side of this plane (Kilpatrick *et al.*[56]). The average positions of the bonds joining groups to the ring will therefore be symmetrical with respect to this plane. Thus in cyclopentane the molecule possesses an effective plane of symmetry in the plane of the ring. We may assume that this state of affairs will remain true in cyclopentane derivatives provided that the groups attached

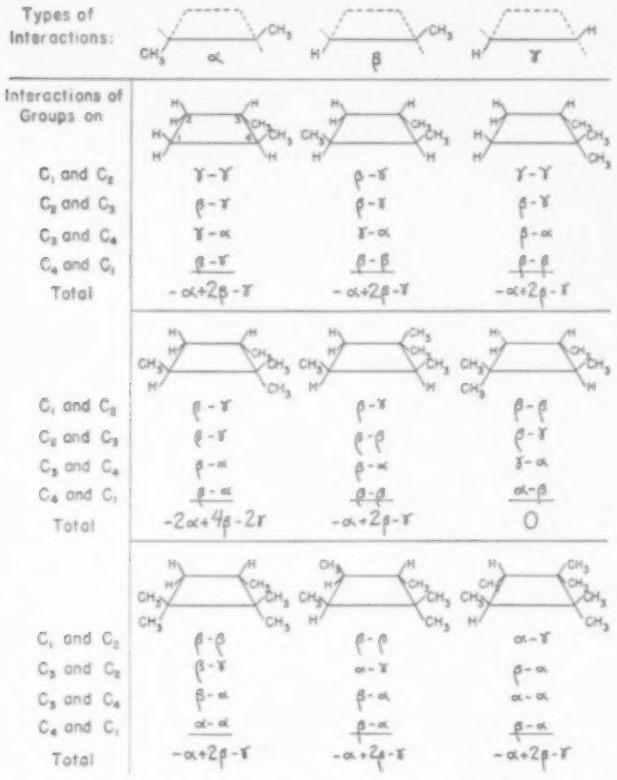


FIG. 12. Application of the principle of pairwise interactions to the nine optically active methyl derivatives of cyclobutane.

to the ring are not too bulky and that they do not approach too closely to one another as the ring undergoes its twisting motions from one conformation to another. This assumption will be far from true if the cyclopentane ring is fused to other rings, especially in a trans conformation.

Starting with this assumption it is readily shown that the optical rotations of the 35 non-enantiomorphic optically active methylated derivatives of cyclopentane ought to be related to one another as shown in Table 5. We see that all of these rotations should be expressible in terms of but two empirical constants (viz., the rotations of *trans*-1,2-dimethyl cyclopentane and *trans*-1,3-dimethyl cyclopentane).

The optical rotations of derivatives of cyclohexane are more complicated to discuss

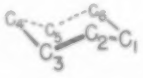
[56] J. E. Kilpatrick, K. S. Pitzer and R. Spitzer, *J. Amer. Chem. Soc.* **69**, 2483 (1947).

TABLE 5. OPTICAL ROTATIONS OF THE METHYL CYCLOPENTANES

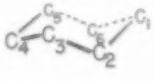
Compound*	$[M]_D$	Compound*	$[M]_D$
1/2 dimethyl	A	1,2/2,3,4 pentamethyl	2A + B
1/3 dimethyl	B	1,2,3,4/2 pentamethyl	−B
1,2/2 trimethyl	A	1,1,2,3,4 pentamethyl	A
1,3/3 trimethyl	B	1,4/1,2,3 pentamethyl	B
1,2/3 trimethyl	A + B	1,2,4/1,2,5 hexamethyl	−B
1,4/2 trimethyl	A − B	1,2,5/1,2,3 hexamethyl	2A + B
1,3/1,2 tetramethyl	−B	1,2,3/1,4,5 hexamethyl	−A − B
1,2/2,3 tetramethyl	2A + B	1,4/1,2,3,5 hexamethyl	B
1,2/3,4 tetramethyl	A + B	1,5/1,2,3,4 hexamethyl	A
1,2,4/3 tetramethyl	B	1,3,4/1,4,5 hexamethyl	A
1,2,3/4 tetramethyl	A	1,3,4,5/1,4 hexamethyl	A − B
1,4/1,2 tetramethyl	A	1,2,3/1,2,3,4 heptamethyl	A
1/1,2,4 tetramethyl	A − B	1,3,4/1,3,4,5 heptamethyl	B
1,2,3/1,2 pentamethyl	−A − B	1,2,5/1,2,3,4 heptamethyl	A + B
1,3,4/1,2 pentamethyl	−B	1,3,4/1,2,4,5 heptamethyl	A − B
1,3/1,2,4 pentamethyl	A − 2B	1,2,3,5/1,2,3,4 octamethyl	A
1,3,5/1,2 pentamethyl	A	1,2,3,4/1,3,4,5 octamethyl	B
1,3/1,2,5 pentamethyl	−A		

* The configurations of the methyl groups are indicated in the same fashion as are those of the hydroxyl groups in Table 1B.

because the ring can exist in two stable chair conformations. Using the notation of Reeves[47,57] these will be denoted by the symbols 1C and C1:



C1



1C

It will be seen that in conformation C1 the axial groups at C_1, C_3 and C_5 point downward and the axial groups at C_2, C_4 and C_6 point upward, whereas in conformation 1C the axial groups at C_1, C_3 and C_5 point upward and those at C_2, C_4 and C_6 point downward. The axial groups in conformation 1C become equatorial groups in C1 and *vice versa*. Since the axial bonds are all parallel, the contributions to the optical rotation by pairwise interactions between any pair of symmetrical axial groups, such as a halide or a methyl or a freely orienting hydroxyl, must vanish. Furthermore, equatorial groups at ring atoms 1 and 3, or 2 and 4, or 3 and 5, or 4 and 6, or 5 and 1, are coplanar, so that the contributions by any pair of equatorial groups at these positions must also vanish. It is found that, as a consequence of these geometrical simplifications, the optical rotations of all polymethyl cyclohexanes should be expressible in terms of two empirical constants—namely the rotation of the conformer of *trans*-1,2-dimethyl cyclohexane in which both methyl groups are equatorial, and the rotation of either of the two chair conformers of *trans*-1,3-dimethyl cyclohexane (both conformers of the latter turn out to have the same rotation, according to the principles set forth here).

It is interesting to consider these conclusions in the light of the known rotations of

[57] R. E. Reeves, *Advanc. Carbohyd. Chem.* **6**, 107 (1951).

the polyhydroxy cyclohexanes, as summarized in Table 6. If the hydroxyl groups in these compounds exist with equal probability in the three equilibrium positions about each carbon-oxygen bond, they can be treated as geometrically equivalent to methyl groups. The rotations calculated using this assumption for each of the chair forms of the known optically active hydroxy cyclohexanes are shown in Table 6. The observed rotations for these compounds are shown in the Table, and it is seen that these rotations are impossible to reconcile with those expected if the two chair forms were in all instances present in equal amounts. (For instance, if 1,2,4/3,5,6 hexitol existed as a mixture of equal amounts of the two chair forms, it should have no optical rotation due to pairwise interactions, yet this compound has the largest rotation of the entire series.) On the other hand, quite good agreement is obtained if we accept Whiffen's assumptions that (1) only that chair form will be present which has the fewest axial hydroxyl groups, and (2) the optical rotation of *trans*-1,3-cyclohexanediol can be neglected in comparison with that of *trans*-1,2-cyclohexanediol. The appreciable rotation of the 1,3,4/2,5 pentitol indicates, however, that the rotation of the 1/3 diol may be of the order of 20 per cent of the rotation of the 1/2 diol.

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It would be most interesting to have measurements of the rotations of these compounds at elevated temperatures in inert solvents. We should expect that the rotations of 1/3-cyclohexanediol and 1,3,4/2,5 cyclohexane-pentitol would be small and independent of temperature, whereas all of the other rotations should decrease in magnitude with temperature as the two chair forms tended to occur in equal amounts. The rotation of 1,3,4/2,5 pentitol should change sign at high temperatures. Unfortunately the data at present available do not provide a very sensitive test for the principle of pairwise interactions, since it is not known to what extent the observed deviations from the predicted values are caused by non-axial symmetry of the hydroxyl groups, by violations of Whiffen's assumptions, or by interactions with the solvent. These data do, however, suggest that the hydroxyl groups in these compounds must be nearly symmetrical, and that one or the other of the two chair forms is rather strongly preferred at room temperature.

The substitution of a carbon in a cyclohexane ring by an oxygen atom should not greatly alter the geometry of the ring. The six-membered pyranose rings of the sugars should therefore exist in two chair forms that resemble those of cyclohexane, and the vicinal actions between hydroxyl groups attached to the pyranose ring should not be very different from the vicinal actions between hydroxyl groups located at equivalent positions on the cyclitols. It is interesting to compare the shifts in rotation that are tabulated in Tables 3 and 4 with the shifts that would be expected in the light of the discussion of the cyclitols.

Let us say that it is carbon atom C_6 in the ring that is replaced by oxygen. A complication arises because this substitution makes it possible for contributions to the optical rotation to arise from interactions between the ring and groups attached to the ring at positions C_1, C_2, C_4 and C_5. The complication can be avoided for the moment if we consider at first only those groups that are attached to the ring at position C_3, since the interactions between the pyranose ring and a freely rotating hydroxyl group at C_3 cannot make any contribution to the optical rotation. Therefore the change in optical rotation that results from a shift in the hydroxyl group from the D to the L configuration at C_3 should involve the same interactions as those that occur in the cyclitols. The discussion is greatly simplified if we assume that the groups on C_3

TABLE 6. OPTICAL ROTATIONS OF SOME CYCLITOLS

Compound	Conformation	No. axial OH groups		Calculated rotation	Calculated rotation based on:*		Calculated by Whiffen[4]	Observed rotation†
		Above ring	Below ring		Assumption I	Assumption II		
1/2 diol	1C1	0	0	A	A/2	A	−45	−48
	4C1	1	1	O				
1/3 diol	1C1	0	1	B	B	B	0	—
	4C1	1	0	B				
1,2/3 triol	1C1	1	1	−A + B	A/2 + B	2A + B	−90	−92
	4C1	1	0	2A + B				
1,2,3/4 tetrol	1C1	1	0	A + B	A/2 + B	A + B	−45	−49
	4C1	2	1	B				
1,2,4/3 tetrol	1C1	2	1	−A + B	B	A + B	−45	−57
	4C1	1	0	A + B				
1,3/2,4 tetrol	1C1	0	0	A	A/2	A	−45	−43
	4C1	2	2	O				
1,2/3,4 tetrol	1C1	1	1	−2A + 2B	A/2 + 2B	‡	‡	−110
	4C1	1	1	3A + 2B				
1,2,4/3,5 pentitol	1C1	2	2	−A	A/2	2A	−90	−91
	4C1	1	0	2A				
1,2,3,5/4 pentitol	1C1	1	0	B	B	B	0	−9
	4C1	3	1	B				
1,3,4/2,5 pentitol	1C1	1	1	−A − B	A/2 − B	− A − B	+45	+41
	4C1	2	1	2A − B				
1,2,4/3,5,6 hexitol	1C1	2	2	−3A	O	3A	−135	−117
	4C1	1	1	3A				

* Assumption I: Conformations 1C and C1 present in equal amounts.
Assumption II: Only that conformation is present which has the fewest axial groups. Whiffen's calculation was based on this assumption; Whiffen also assumed A = −45°, B = 0.

† Data from Angyal and Anderson[58], Wilson and Read[58], Posternak, Friedli and Reymond[59,60] and Posternak and Reymond[61].

‡ Since both chair forms have the same number of axial groups, one would expect a rotation of A/2 = −23° using Whiffen's results. If C1 alone were present one would expect a rotation of −2A = +90°, and if 1C alone were present the rotation would be 3A = −135. The observed rotation of −110° would indicate that the substance is predominantly in the 1C conformation but it is not readily evident why this should be so.

[58] N. A. B. Wilson and J. Read, *J. Chem. Soc.* 1269 (1935).
[59] T. Posternak, H. Friedli and D. Reymond, *Helv. Chim. Acta* **36**, 251 (1953).
[60] T. Posternak, H. Friedli and D. Reymond, *Helv. Chim. Acta* **38**, 205 (1955).
[61] T. Posternak and D. Reymond, *Helv. Chim. Acta* **38**, 195 (1955).

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interact predominantly with groups that are located on the adjacent carbon atoms, C_2 and C_4; this means that we shall follow Whiffen in assuming that the interactions involved in the constant B of Table 6 will be neglected. We have seen that this assumption leads to good results with the cyclitols, even though it cannot be exactly correct. Under these conditions it is readily seen that, provided the ring conformation remains fixed as either Cl or 1C, a change in configuration at $C_2C_3C_4$ from DDD to DLD or from LDL to LLL should result in no change in optical rotation. Unfortunately there is only one example with which to test this production, namely the change in rotation in going from β-D-glucose to β-D-allose, which amounts to $-34°$.

The failure of this rotation change to vanish might be explained in several ways. Perhaps the interactions between the groups at C_3 with the groups at C_1 and C_5 are not negligible. It seems, however, unlikely that these interactions would be as large as 34°, in view of the considerably smaller upper limit to the value of B in the cyclitols. An alternative possibility is that β-D-glucose and β-D-allose do not have the same chair conformation. This disagrees with the considerations of Reeves[47], which indicate that both sugars are probably in the Cl conformation. (The correctness of Reeves' views also gains support in what follows.) The only other explanation that seems plausible (aside, perhaps, from an error in the rotation of β-D-allose) is that in one of these two compounds—probably β-D-allose—there is for some reason a restricion in the ability of the hydroxyl groups to assume axial symmetry about the bonds that hold them to the ring.

It is also readily seen that, again assuming that the constant B in Table 6 can be neglected, a change in configuration at $C_2C_3C_4$ from LDD to LLD will produce a change in rotation of $-3A$ if the ring conformations for both configurations is Cl, and $+3A$ if the ring conformations are both 1C, A being the same constant as that appearing in Table 6. Furthermore, a change from DDL to DLL at these positions will produce a rotation change of $+3A$ if the rings are in the Cl conformation, and $-3A$ if they are in the 1C conformation.

Reeves[47] has given reasons for believing that the Cl conformation is almost exclusively present in the α and β forms of D-glucose, D-galactose, D-mannose, D-gulose, D-talose, D-ribose, D-xylose and L-arabinose. The pyranose ring of α-D allose is also believed to exist in the Cl conformation. On the other hand, α- and β-D-altrose, D-idose and D-lyxose, and β-D-allose probably do not exist exclusively in the Cl conformation.

The following rotation changes are found in Table 4 for pairs of sugars and sugar derivatives which are believed to exist in the Cl conformation, and which have the configurations DDL and DLL at $C_2C_3C_4$:

α-D-gulose, α-D-galactose	$-157°$
β-D-α-glucoheptose, β-D-α-mannoheptose	$-157°$
Methyl α-D-guloside, methyl α-D-galactoside	$-148°$
Methyl β-D-guloside, methyl β-D-galactoside	$-162°$
Methyl tetracetyl α-D-guloside, methyl tetracetyl α-D-galactoside	$-130°$
Methyl tetracetyl β-D-guloside, methyl tetracetyl β-D-galactoside	$-66°$

(D-α-glucoheptose and D-α-mannoheptose have pyranose rings in which the hydroxyl

configurations are identical with those in D-gulose and D-galactose, respectively.) If we take Whiffen's value of $-45°$ for A, then a rotation change of $+3A = -135°$ would be expected in these compounds. This is not very different from the observed values, even in the methyl glycosides, and surprisingly enough the correct sign and the correct order of magnitude are obtained even for the acetylated methyl glycosides, whose rotations were measured in chloroform.

The conformation change LDD to LLD at $C_2C_3C_4$ brings about the following rotation changes:

β-D-altrose, β-D-mannose	$+90°$
β-celtrobiose, β-(4-β-glucosido)-D-mannose	$+71°$
Methyl tetramethyl α-D-altroside, methyl tetramethyl α-D-mannoside	$+137°$

(Celtrobiose is 4-(β-glucosido)-D-altrose.) The predicted rotation change for sugars in the Cl conformation is $-3A = +135°$. In spite of the fact that D-altrose is not believed to exist exclusively in the Cl conformation, the observed shifts in rotation are correct in sign and not far off in order of magnitude.

Similar considerations can be applied to the changes in rotation that result when configurations are changed at positions C_1 and C_2 in the pyranose sugars. Let us assume that when the ring is in the conformation Cl a change in the configuration at C_2 from D to L produces a change R_2 in the contribution to the optical rotation arising from the interaction between the groups at C_2 and the ring. Then it is easily seen that a change in configuration at $C_1C_2C_3$ fromDDD to DLD or from LDL to LLL should produce a change in rotation equal to R_2, provided, as before, that we neglect interactions of the type that lead to the quantity B in Table 6. The observed rotation changes are as follows (changes which involve sugars not believed to be exclusively in the Cl conformation are given in brackets):

DDD → DLD:

[Methyl α-D-guloside, methyl α-D-iodoside	$+31°$]

LDL → LLL:

β-D-glucose, β-D-mannose	$+64°$
β-D-galactose, β-D-talose	$+71°$
[α-L-arabinose, α-L-ribose	$+85°$]
β-cellobiose, β-(4-β-glucosido)-D-mannose	$+71°$
Methyl β-D-glucoside methyl β-D-mannoside	$+68°$
Methyl β-D-glucomethyloside, methyl β-D-rhamnoside	$+72°$
[Methyl β-D-xyloside, methyl β-D-lyxoside	$+102°$]
Phenyl β-D-glucoside, phenyl β-D-mannoside	$0°$
[α-L-arabinose tetracetate, α-L-ribose tetracetate	$+308°$]
β-D-glucose pentacetate, β-D-mannose pentacetate	$+113°$
β-cellobiose octacetate, β-(4-β-D-glucosido)-mannose octacetate	$-14°$
Methyl 2,3,4,6 tetramethyl β-D-glucose, methyl 2,3,4,6 tetramethyl α-D-mannose	$+157°$

The results show that R_2 has a value of about $+70°$; all of the observations that differ appreciably from this value involve compounds that are not exclusively in the Cl conformation or are heavily acetylated or methylated.

A change at $C_1C_2C_3$ from DDL to DLL results in the following changes in rotation (as above, changes involving sugars not believed to be exclusively in the Cl conformation are given in brackets):

[α-D-xylose, α-D-lyxose	+132°
α-D-glucose, α-D-mannose	+149°
α-D-galactose, α-D-talose	+149°
α-L-β-guloheptose, α-L-α-guloheptose	+157°
α-D-α-mannoheptose, α-D-β-mannoheptose	+170°
α-D-glucomethylose, α-D-rhamnose	+104°
[Methyl β-L-arabinoside, methyl β-L-riboside	+228°]
[Methyl α-D-xyloside, methyl α-D-lyxoside	+154°]
Methyl α-D-glucoside, methyl α-D-mannoside	+148°
Methyl α-cellobiose, methyl α-(4-β-glucosido)-D-mannose	+181°
Methyl α-D-glucomethyloside, methyl α-D-rhamnoside	+153°
Phenyl α-D-glucoside, phenyl α-D-mannoside	+171°
Various acetates	+46° to +283°
Various haloacetates	+106° to +291°

A consideration of the molecular geometry in the Cl conformation shows that one would predict a rotation change of $R_2 - 3A$ for this group of compounds. Setting $R_2 = +70°$ and $A = -45°$, this gives a predicted change of +205°—somewhat greater than is observed, but of the correct sign and order of magnitude, even for the methyl and phenyl glycosides and for the methylated and acetylated derivatives.

For a sugar in the Cl conformation a configuration change from LDD to LLD at $C_1C_2C_3$ would be expected to produce a change in rotation of $R_2 + 3A = +70° - 135° = -65°$. The examples listed in Table 4 all involve sugars that are not entirely in the Cl conformation, but in spite of this the values are quite close to the expected one:

[β-D-allose, β-D-altrose	−59°]
[β-D-α-glucoheptose, β-D-β-glucoheptose	−60°]
[Methyl β-D-guloside, methyl β-D-idoside	−68°]

It is also possible to interpret the changes in optical rotation accompanying a configuration change at position C_1, the glycosidic carbon atom. A hydroxyl group at C_1 will, according to the assumptions made in these paragraphs, contribute to the rotation (a) through its interaction with the ring, and (b) through its interaction with the groups at position C_2. Let R_1 denote the contribution of the ring interaction (a) to the change in rotation when a hydroxyl at C_1 is converted from the D configuration to the L configuration, the ring conformation being Cl. Consideration of the ring geometry in the usual manner shows that if the ring conformation is Cl and the hydroxyl at C_2 is in the D configuration, then interaction (b) will change by $-2A$ when the hydroxyl at C_1 is changed in configuration from D to L (i.e. from α to β in the more usual nomenclature of groups at the glycosidic carbon atom). On the other hand, if the hydroxyl at C_2 is L and the ring conformation is Cl,

then interaction (b) will change by $+A$ when the configuration at C_1 is changed from D to L. Thus we see that if the configuration at atoms C_1C_2 is changed from DD to LD in a sugar whose ring is in the Cl conformation, the optical rotation should change by $R_1 - 2A$, whereas a configuration change at the same positions from DL to LL will change the rotation by $R_1 + A$. As seen in Table 3, free sugars in the "glucose class" undergo a configuration change of the former type (DD to DL) in transforming from the α to the β form, and the accompanying change in optical rotation is about $+175°$ for the free sugars. Sugars in the "mannose class" undergo a configuration change of the DL to LL type in going from the α form to the β form, and the optical rotation change (where both forms are in the Cl ring conformation) is about $+80°$. Thus we expect that

$$R_1 - 2A = +175°$$

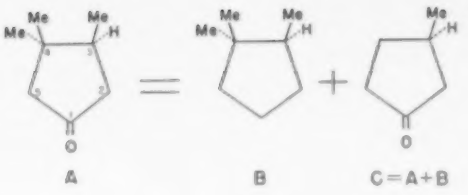
$$R_1 + A = +80°$$

which leads to the results, $R_1 = +112°$, $A = -32°$. This value of A is in only fair agreement with that obtained from the study of the cyclitols (viz., $A = -45°$). It is interesting to note that, in contrast with the findings of many other sugar derivatives in Table 4, groups other than free hydroxyls (e.g. methoxy, phenoxy and acetyl) give considerably different values of A from that just found with the unsubstituted sugars.

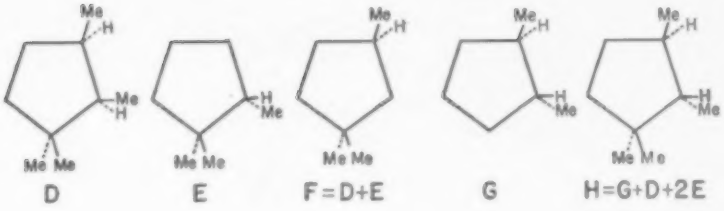
In summary, it appears that the rotation changes in the sugars are at least roughly consistent with the observed rotations of the cyclitols, indicating that the interactions between hydroxyl groups are similar in magnitude, though not exactly the same. It is evident that the principle of pairwise interactions cannot lead to very accurate predictions of the rotations of the sugars, possibly because of restricted rotation of the hydroxyl groups and solvation effects that vary irregularly with configuration.

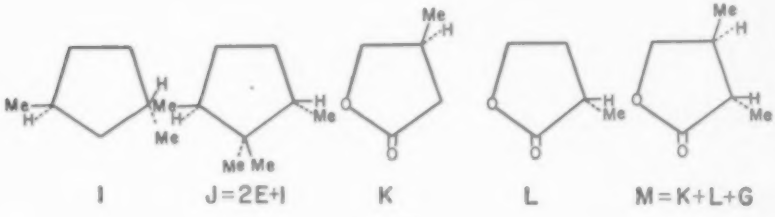
Fig. 13 shows a number of further relationships between the optical rotations of compounds containing five-membered rings. These relationships are all derived assuming that the ring has an effective plane of symmetry in the plane of the ring, as previously discussed. Figs. 14 and 15 show relationships involving some compounds with fused five- and six-membered rings.

Many bicyclo compounds have the advantage that the ring systems are relatively rigid, so that uncertainties arising from the possibility of several ring conformations, or even from distortions of otherwise stable symmetrical ring conformations, do not arise. Numerous relationships may be deduced for the optical rotations of such compounds, and it is probably here that the best tests of the principle of pairwise interactions should be sought. For instance, in the dilactones A and B shown in Fig. 16 the optical rotations should be independent of the nature of the groups R_1, R_2, R_3 and R_4 so long as these groups are axially symmetric (H, CH_3, halide or CN) or so long as they can acquire axial symmetry by rotation about the bonds joining them to the bridgehead carbon atoms (ethyl, hydroxyl). This results from the fact that none of the pairwise interactions between either R_1 or R_2 and the rest of the molecule, or between R_1 and R_2 themselves, can result in any contribution to the optical rotation, since each pair of groups involving R_1 or R_2 as one member of the pair either may be superimposed on its own mirror image or it is counteracted by another pair which bears a mirror image

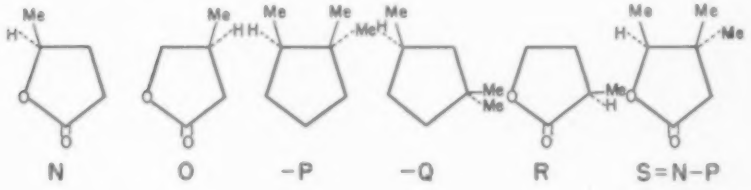


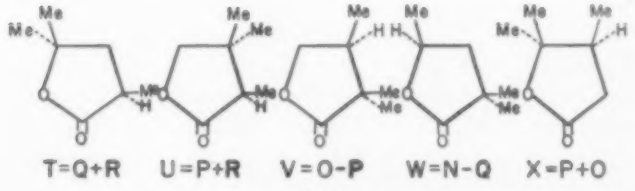
13 (a)





13 (b)





13 (c)

FIG. 13a,b,c. Relationships between the optical rotations of compounds containing five-membered rings.

relationship to it. In the compounds C, D, E and F in Fig. 16 it is easily seen that the rotations should be related by

$$C = D = \tfrac{1}{2}(E + F)$$

where the R_1 are any axially symmetric groups.*

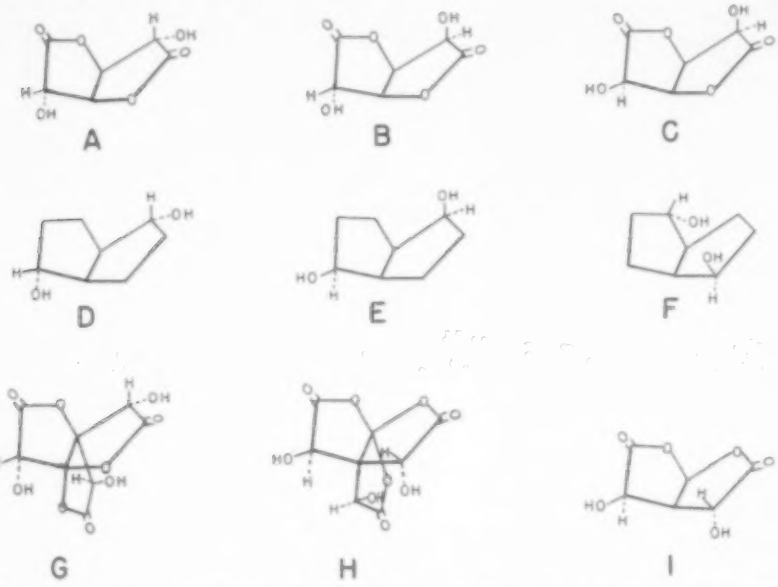


FIG. 14. Compounds containing fused five-membered rings. If the principle of pairwise interactions is valid, one should expect the following relationships:

$$A + C = 2B + D + E + F$$
$$A + I = B + G + H$$

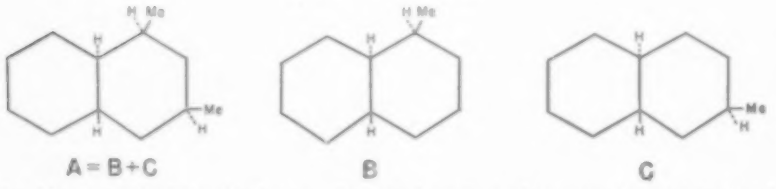


FIG. 15. Expected relationship between the optical rotations of compounds containing fused six-membered rings.

The bicyclo compounds related to camphor offer a particularly fruitful area for the application of the principle of pairwise interactions because their rigidly cross-braced frame-work produces a well defined and fairly symmetrical structure which should not change appreciably when groups are substituted on it at different positions. Furthermore, the optical rotations of many of these compounds are known, so that some of the expected relationships may be tested.

* Turner, Meador and Winkler[62] have pointed out that these bicyclo compounds are not entirely rigid, but that the upper half of the molecule may twist through an appreciable angle relative to the lower half without distortion of the bond angles. Mr. F. Fong[63] has shown, however, that when account is taken of the barrier to internal rotation about the carbon-carbon bonds in this molecule, the symmetrical, untwisted form is probably the most stable. J. J. MacFarlane and I. G. Ross (*J. Chem. Soc.* 4169 (1960) have also shown from infra-red and Raman spectra that the molecule is probably not twisted.

[62] R. B. Turner, W. R. Meador and R. E. Winkler, *J. Amer. Chem. Soc.* **79**, 4121 (1957).
[63] F. Fong, personal communication.

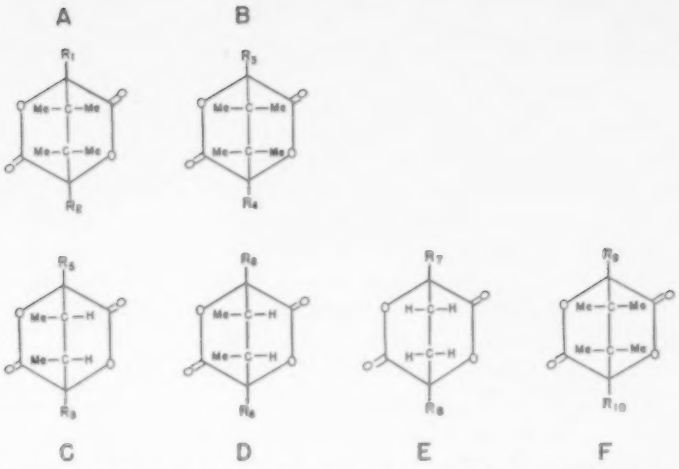


FIG. 16. Compounds containing fused ring systems. See text for relationships between the optical rotations.

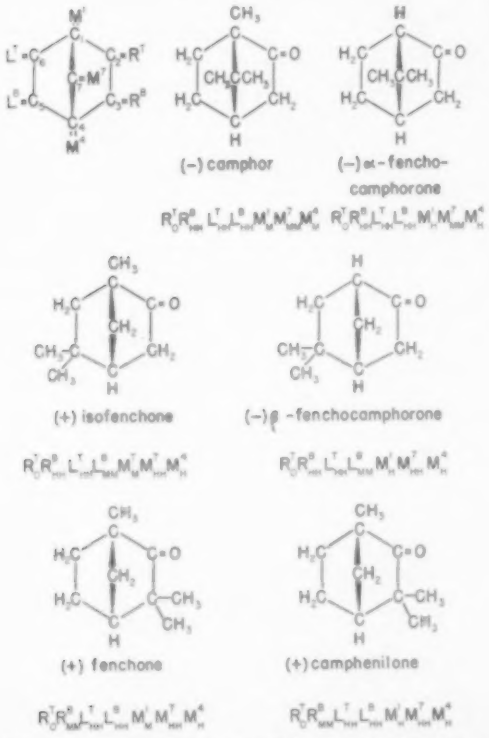


FIG. 17. Nomenclature for derivatives of dimethyl and trimethyl [1.2.2] bicycloheptanes.

The derivation of relationships between the rotations of compounds of this type is facilitated by adopting a simple notation. The positions of the seven ring carbon atoms in the bicyclo [1.2.2] heptanes are given the symbols shown in Fig. 17, R^6 referring to the atom on the right that lies toward the top of the paper (generally designated as C_2),

R_b referring to the atom on the right that lies toward the bottom (generally designated as C_2), M^1, M^4 and M^7 referring to the atoms C_1, C_4 and C_7 that lie in the mid-plane of the molecule and L^t and L^b referring to the atoms C_5 and C_6 that lie on the left and, respectively, toward the top and bottom of the page. It will be assumed that the framework has a configuration such that, if carbon atoms C_2, C_3, C_5 and C_6 are in the plane of the page, then C_1, C_4 and C_7 lie above the page, toward the reader. These symbols are in addition given subscripts which describe the atoms or groups that are attached to the corresponding positions in the ring: O for an oxygen atom, as in a ketone, M for a methyl group, MM for two methyl groups, HH for two hydrogen atoms, etc. Note that only one monovalent group can be attached to the bridgehead positions, M^1 and M^4, whereas two monovalent groups or one bivalent group may be attached elsewhere. The various derivatives of bicyclo [1.2.2] heptane can then be indicated by symbols as follows. Levorotatory camphor is $R^t_O R^b_{HH} L^t_{HH} L^b_{HH} M^1_M M^7_{MM} M^4_H$, and dextrorotatory fenchone is $R^t_O R^b_{HH} L^t_{HH} L^b_{MM} M^1_M M^7_{HH} M^4_H$ (see Fig. 17 for structural formulae).

According to the principle of pairwise interactions the optical rotation is the sum of the effects of the different parts of the molecule on each other. For instance, in camphor the group of atoms symbolized by M^1_M (the upper bridgehead with its methyl group) interacts with the group of atoms symbolized by R^t_O (the carbonyl group at position 2) to produce a contribution to the optical rotation that will be denoted by $[M^1_M, R^t_O]$. The optical rotation of (−) camphor* will be the sum of all interactions between the seven symbols that represent camphor:

$$\begin{aligned}[M] = {} & [R^t_O, R^b_{HH}] + [R^t_O, L^t_{HH}] + [R^t_O, L^b_{HH}] + [R^t_O, M^1_M] + [R^t_O, M^7_{MM}] + [R^t_O, M^4_H] \\ & + [R^b_{HH}, L^t_{HH}] + [R^b_{HH}, L^b_{HH}] + [R^b_{HH}, M^1_M] + [R^b_{HH}, M^7_{MM}] + [R^b_{HH}, M^4_H] \\ & + [L^t_{HH}, L^b_{HH}] + [L^t_{HH}, M^1_M] + [L^t_{HH}, M^7_{MM}] + [L^t_{HH}, M^4_H] + [L^b_{HH}, M^1_M] \\ & + [L^b_{HH}, M^7_{MM}] + [L^b_{HH}, M^4_H] + [M^1_M, M^7_{MM}] + [M^1_M, M^4_H] + [M^7_{MM}, M^4_H]\end{aligned}$$

It should be pointed out that although the symbol [A,B] does not correspond to ordinary multiplication of the quantities A and B, it does possess some of the characteristics of ordinary multiplication. For instance it is true that

$$[A,B] = [B,A]$$

for any pair of symbols A and B. It is also possible to define the operation

$$[A,(B + C)] = [A,B] + [A,C]$$

This permits us to write the pairwise contributions to the optical rotation of camphor in the somewhat more compact form

$$\begin{aligned}[M] = {} & [R^t_O, (R^b_{HH} + L^t_{HH} + L^b_{HH} + M^1_M + M^7_{MM} + M^4_H)] \\ & + [R^b_{HH}, (L^t_{HH} + L^b_{HH} + M^1_M + M^7_{MM} + M^4_H)] \\ & + [L^t_{HH}, (L^b_{HH} + M^1_M + M^7_{MM} + M^4_H)] + [L^b_{HH}, (M^1_M + M^7_{MM} + M^4_H)] \\ & + [M^1_M, (M^7_{MM} + M^4_H)] + [M^7_{MM}, M^4_H]\end{aligned}$$

* The absolute configurations of camphor and other bicyclo derivatives considered here have been assigned by a study of the reactions through which these substances may be interconverted (Hückel[64]; Clough[65]; Jacob *et al.*[66]).

[64] W. Hückel, *J. Prakt. Chem.* **157**, 225 (1941).
[65] F. B. Clough, Ph.D. Dissertation, Princeton University (1950).
[66] G. Jacob, G. Ourisson and A. Rassat, *Bull. Soc. Chim. Fr.* 1374 (1959).

Further simplifications are possible because certain of the pairwise contributions vanish as a result of the symmetry of the molecule. This is true of all of the interactions of M^1_M, M^7_{MM} and M^t_H with each other (that is, all of the interactions among the atoms in the portion CH_3—C—$C(CH_3)_2$—CH of the camphor molecule), since all of the pairs of atoms and groups concerned are superimposable on their mirror images. The interactions $[L^t_{HH},L^b_{HH}]$, $[R^t_{HH},L^t_{HH}]$, $[R^b_{HH},L^b_{HH}]$, $[R^t_{HH},R^b_{HH}]$, and $[R^t_O,R^b_{HH}]$, also make no contribution to the optical rotation. Furthermore, certain interacting pairs are mirror images of other interacting pairs, so their contributions to the optical rotation differ only in sign. Thus

$$[M^1_M,R^b_{HH}] = -[M^1_M,L^b_{HH}]$$

$$[R^b_{HH},L^t_O] = -[L^b_{HH},R^t_O]$$

$$[R^t_{HH},L^b_{HH}] = -[R^b_{HH},L^t_{HH}]$$

$$[R^t_O,L^t_{HH}] = -[L^t_O,R^t_{HH}]$$

$$[R^b_{HH},L^b_{MM}] = -[L^b_{HH},R^b_{MM}]$$

When these simplifications are taken into account we find that the pairwise contributions to the rotation of (−) camphor are given by

$$[M] = [R^t_O,L^b_{HH}] + [R^b_{HH},L^t_{HH}] + [R^t_O,L^t_{HH}] + [(L^t_{HH} + R^t_O)(M^1_M + M^7_{MM} + M^t_H)]$$

Similar reasoning can be applied to the remaining compounds illustrated in Fig. 17, and the resulting expressions for the optical rotations are given in Table 7. Inspection of this table shows that the following relationships between the optical rotations should exist. (The optical rotations are represented by [I] for (−) camphor, [II] for (−)α fencho-camphorone, etc., the Roman numerals being those in the left hand column of Table 7).

$$[I] - [II] = [(L^t_{HH} + R^t_O),(M^1_M - M^1_H)]$$

$$[III] - [IV] = [(L^t_{HH} + R^t_O + L^b_{MM} + R^b_{HH}),(M^1_M - M^1_H)]$$

$$[V] - [VI] = [(L^t_{HH} + R^t_O + L^b_{HH} + R^b_{MM}),(M^1_M - M^1_H)]$$

The symmetry of bicyclo [1.2.2] heptane is such that the pair $R^b_{MM}M^1_M$ and the pair $L^b_{MM}M^1_M$ are mirror images of each other, and the same is true of $R^b_{MM}M^1_H$ and $L^b_{MM}M^1_H$. Therefore $[R^b_{MM},M^1_M] = -L^b_{MM},M^1_M]$ and $[R^b_{MM},M^1_H] = -[L^b_{MM},M^1_H]$. Consequently we arrive at the relation

$$[I] - [II] = \tfrac{1}{2}\{([III] - [IV]) + ([V] - [VI])\}$$

The observed rotations of these compounds in various solvents are shown in Table 7. Unfortunately all of the rotations have not been measured in an inert solvent, nor have they even been measured in a common solvent, and it is evident that rather large solvent effects exist. Taking the extreme values of the observed rotations of each compound in all of the solvents in which measurements have been made we find that

$$[I] - [II] \text{ lies between } -40° \text{ and } -68°$$

$$\tfrac{1}{2}\{([III] - [IV]) + ([V] - [VI])\} \text{ lies between } -10{\cdot}5° \text{ and } +42{\cdot}5°$$

TABLE 7. OPTICAL ROTATIONS OF SOME CONFIGURATIONALLY RELATED BICYCLO-KETONES

Compound	Expression for optical rotation	$[M]_D$	Solvent
I. (−)camphor	$R^b_{HH}L^t_{HH} + R^t_OL^b_{HH} + R^t_OL^t_{HH} + (R^t_O + L^t_{HH})(M^t_M + M^7_{MM} + M^4_H)$	−88	hexane
		−87	ether
		−75, −77	acetone
		−68	CS_2
		−63, −65	ethanol
		−62	methanol
		−58, −61	benzene
II. (−)α-fencho-camphorone	$R^b_{HH}L^t_{HH} + R^t_OL^b_{HH} + R^t_OR^t_{HH} + (R^t_O + L^t_{HH})(M^t_M + M^7_{MM} + M^4_H)$	−20	ether
III. (+)isofenchone	$R^b_{HH}L^t_{HH} + R^t_OL^b_{MM} + R^t_OL^t_{HH} + R^b_{HH}L^b_{MM} + (R^t_O + R^b_{HH} + L^t_{HH} + L^b_{MM})(M^t_M + M^7_{HH} + M^4_H)$	+13, 15	undiluted
		+10	ethanol
IV. (−)β-fencho-camphorone	$R^b_{HH}L^t_{HH} + R^t_OL^b_{MM} + R^t_OL^t_{HH} + R^b_{HH}L^b_{MM} + (R^t_O + R^b_{HH} + L^t_{HH} + L^b_{MM})(M^t_H + M^7_{HH} + M^4_H)$	−23	ether
		+10	ethanol
V. (+) fenchone	$R^b_{MM}L^t_{HH} + R^t_OL^b_{HH} + R^t_OL^t_{HH} + R^b_{MM}L^b_{HH} + (R^t_O + R^b_{MM} + L^t_{HH} + L_{HH})(M^t_M + M^7_{HH} + M^4_H)$	+115	benzene
		+104, 110	ethanol
		+95, 102	undiluted
		+91	methanol
		+86, 89	cyclohexane
		+76	ether
VI. (+)camphenilone	$R^b_{MM}L^t_{HH} + R^t_OL^b_{HH} + R^t_OL^t_{HH} + R^b_{MM}L^b_{HH} + (R^t_O + R^b_{MM} + L^t_{HH} + L_{HH})(M^t_H + M^7_{HH} + M^4_H)$	+97	ethanol
		+85, 89	benzene
		+75	ether
		+70	cyclohexane

If we restrict ourselves to the solvent diethyl ether (except for isofenchone, where the averaged values in the undiluted state and in alcohol will be used) we find

$$[I] - [II] = -67°$$

$$\tfrac{1}{2}\{([III] - [IV]) + ([IV] - [VI])\} = +18{\cdot}6°$$

If only the measurements in ethanol are used (except for α-fenchcamphorone, where the only available measurement is in ether),

$$[I] - [II] = -44°$$

$$\tfrac{1}{2}\{([III] - [IV]) + ([V] - [VI])\} = +5°$$

In none of these methods of treating the data is the agreement with the expected values at all satisfactory.* Whether this is caused by the use of different solvents for the different substances, or whether one or more of the measurements is in error, or whether the principle of pairwise interactions is for some reason not applicable in these

* Jacob *et al.*[66] have indicated that the optical rotations and the rotatory dispersions of all of the compounds listed in Table 7 have been measured in their laboratory and promise that the results will appear in a future publication.

compounds it is not possible to say. It should be emphasized, however, that this set of compounds would be expected to be particularly suitable for testing the principle since the molecular skeletons are rigid. Furthermore, all of the groups involved other than carbonyl are non-polar and the changes that are made in going from one compound to another occur at relatively well separated points, so that the effects of higher order interactions ought to be especially small.

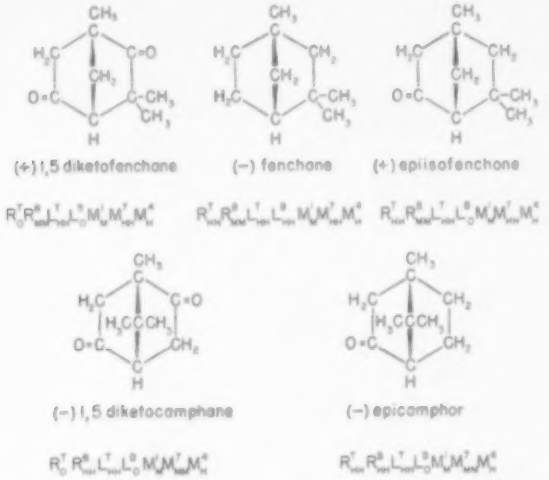


FIG. 18. Methyl ketones of [1.2.2] bicycloheptanes whose rotations should be interrelated as described in the text.

Another group of compounds whose rotations can be shown to be related is shown in Fig. 18 and Table 8. Using the same reasoning as that employed above, and denoting the optical rotations by the bracketed capital letters given in the left hand column of Table 8, one finds that

$$[A] + [B] - [C] - [D] = [R^t_O L^b_O] + [R^t_{HH} L^b_{HH}] - [R^t_O L^b_{HH}] - [R^t_{HH} L^b_O]$$

$$[E] - [F] - [G] = [R^t_O L^b_O] - [R^b_{HH} L^t_{HH}] - [R^t_O L^b_{HH}] - [R^t_{HH} L^b_O]$$

Since $[R^t_{HH} L^b_{HH}] = -[R^b_{HH} L^t_{HH}]$ we should expect that

$$[A] + [B] - [C] - [D] = [E] - [F] - [G]$$

Although the rotations of all of these compounds are available, several solvents have been used and we have no set of rotations in a common solvent. Taking the extreme rotations of each of the compounds in all of the solvents in which they have been measured we find that

$[A] + [B] - [C] - [D]$ takes values between $+3°$ and $-27°$

and

$[E] - [F] - [G]$ takes values between $+7°$ and $-93°$

This result is consistent with the prediction, but the solvent effects are evidently too variable to make a real test possible.

TABLE 8. OPTICAL ROTATIONS OF CONFIGURATIONALLY RELATED BICYCLO-KETONES, DIKETONES AND HYDROCARBONS

Compound	Expression for optical rotation	$[M]_D$	Solvent
A. (+), 1,5-diketo-fenchane	$R^t_O L^b_O + R^b_{MM} L^t_{HH} + R^t_O L^t_{HH} + R^b_{MM} L^b_O + (R^t_O + R^b_{MM} + L^t_{HH} + L^b_O)(M^1_M + M^7_{HH} + M^4_H)$	+126 +125	benzene methanol
B. (−) fenchane	$R^t_{HH} L^b_{HH} + R^b_{MM} L^t_{HH} + R^b_{MM} L^b_{HH} + (R^b_{MM} + L^b_{HH})(M^1_M + M^7_{HH} + M^4_H)$	−25 −23, −25	undiluted ethanol
C. (+) fenchone	$R^b_{MM} L^t_{HH} + R^t_O L^b_{HH} + R^t_O L^t_{HH} + R^b_{MM} L^b_{HH} + (R^t_O + R^b_{MM} + L^t_{HH} + L^b_{HH})(M^1_M + M^7_{HH} + M_H)$	See Table 7	
D. (+) epiisofenchone	$R^t_{HH} L^b_O + R^b_{MM} L^t_{HH} + R^b_{MM} L^b_O + (R^b_{MM} + L^b_O)(M^1_M + M^7_{HH} + M^4_H)$	+21	undiluted
E. (−), 1,5-diketo camphane	$R^t_O L^b_O + R^b_{HH} L^t_{HH} + R^t_O L^t_{HH} + R^b_{HH} L^b_O + (R^t_O + R^b_{HH} + L^t_{HH} + L^b_O)(M^1_M + M^7_{MM} + M^4_H)$	−220 −172, −194 −177 −162	ligroin ethanol ether water
F. (−) camphor	$R^b_{HH} L^t_{HH} + R^t_O L^b_{HH} + R^t_O L^t_{HH} + (L^t_{HH} + R^t_O)(M^1_M + M^7_{MM} + M^4_H)$	See Table 7	
G. (−) epicamphor	$R^b_{HH} L^t_{HH} + R^t_{HH} L^b_O + R^b_{HH} L^b_O + (R^b_{HH} + L^b_O)(M^1_M + M^7_{MM} + M^4_H)$	−96 −89 −69	CS_2 benzene methanol

TABLE 9. RELATIONSHIPS BETWEEN THE OPTICAL ROTATIONS OF THE 3-HALO CAMPHORS (LOWRY[67])

Compound	$[M]_{5461}$ (benzene)
camphor	79·7
3α-chlorocamphor	153·7
3β-chlorocamphor	69·9
α + β − camphor	143·9
3,3-dichlorocamphor	147·3
3α-bromocamphor	324·2
3β-bromocamphor	−101·1
α + β − camphor	143·4
3,3-dibromocamphor	147·6

Lowry[67] prepared the 3α- and 3β-monochlorides and monobromides and the 3,3-dichloride and dibromide of camphor and measured the optical rotations in benzene (Table 9). He demonstrated that the rotations very closely obey the relationship

3α-monohalocamphor + 3β-monohalocamphor − camphor
= 3,3-dihalocamphor

It is easily shown that the observation is a consequence of the principle of pairwise interactions if one makes the same assumptions regarding the geometry of the bicyclo

[67] T. M. Lowry, *Optical Rotatory Power* p. 310. Longmans, Green, London (1935).

[1.2.2] heptane skeleton that have been employed in the previous discussion. Let us introduce the symbols $R^b_{X\alpha}$,$R^b_{X\beta}$,$R^b_{H\alpha}$,$R^b_{H\beta}$ to represent, respectively, X(the halogen atom) in the α configuration, X in the β configuration, H(the hydrogen atom) in the α configuration, and H in the β configuration, at position R^b. The carbon atom in the ring at R^b will be represented by R^b_C. Then writing the symbol [A] for the sum of those pairwise interactions which are common to all of the compounds studied by Lowry,

$$[A] = [R^b_C,L^t_{HH}] + [R^t_O,L^b_{HH}] + [R^t_O,L^t_{HH}] + [R^b_C,L^b_{HH}] + [(R^b_C + R^t_O + L^t_{HH} + L^b_{HH}), (M^1_M + M^7_{MM} + M^4_H)]$$

we find that camphor has the rotation

$$[M] = [A] + [(R^b_{H\alpha} + R^b_{H\beta}),(R^t_O + L^t_{HH} + L^b_{HH} + M^1_M + M^7_{MM} + M^4_H)]$$

The α halogen derivative of camphor as the rotation

$$[M] = [A] + [(R^b_{X\alpha} + R^b_{H\beta}),(R^t_O + L^t_{HH} + L^b_{HH} + M^1_M + M^7_{MM} + M^4_H)]$$

The β halogen derivative has the rotation

$$[M] = [A] + [(R^b_{H\alpha} + R^b_{X\beta}),(R^t_O + L^t_{HH} + L^b_{HH} + M^1_M + M^7_{MM} + M^4_H)]$$

The 3,3-dihalogen derivative has the rotation

$$[M] = [A] + [(R^b_{X\alpha} + R^b_{X\beta}),(R^t_O + L^t_{HH} + L^b_{HH} + M^1_M + M^7_{MM} + M^4_H)]$$

Lowry's relationship follows directly from these expressions.

The success of this relationship here is surprising because these compounds would not be expected to be as favorable for the application of the principle of pairwise interactions as were the bicyclo [1.2.2] heptane derivatives that were previously discussed. The substitution of one or two rather strongly polar halogen atoms for hydrogen atoms on the carbon atom adjacent to the carbonyl group in camphor might be expected to have an appreciable effect on the pairwise interactions of the carbonyl group with the remaining groups in the molecule (i.e., on the terms that go to make up the quantity [A]). Furthermore, one would think that the interaction terms $[R^b_{X\alpha},R^b_O]$ and $[R^b_{X\beta},R^b_O]$ between the halogens at C_3 and the carbonyl might depend considerably on whether the second atom at C_3 is hydrogen or halogen. The success of the principle here makes it all the more likely that the much poorer agreement observed in the previous examples of bicyclo [1.2.2] heptane derivatives must be ascribed either to solvent effects or to erroneous experimental data.

Several other relationships among the optical rotations of derivatives of bicyclo [1.2.2] heptane derivatives are indicated in Fig. 19. These relationships may be derived by the methods illustrated above. Data are not available to test these relationships.

One other group of compounds has been found in which the pairwise interaction principle may be tested. This group is made up of steroids in which ring fusion freezes the rings into conformations which presumably resemble one or the other of the chair forms of cyclohexane. It has been shown above that any pair of axially symmetrical groups whose axes lie in the same plane cannot contribute to the optical rotation by pairwise interactions. Such a relationship exists for any pair of groups attached to a cyclohexane ring at axial (i.e., "polar") positions, and it also exists for any pair of 1,3

TABLE 10. RELATIONSHIPS BETWEEN THE OPTICAL ROTATIONS OF STEROIDS DATA FROM MATHIEU AND PETIT[65]

	$[M]_D$ (in $CHCl_3$)
1. 1α-hydroxy-5α-cholestane	+96
2. 3α-hydroxy-5α-cholestane	+126
3. 5α-cholestane	+91
(1) + (2) − (3)	+131
1α,3α-dihydroxy-5α-cholestane	+101
1. 3α-hydroxy-5α-cholestane	+126
2. 5α-hydroxy-5α-cholestane	+39
3. 5α-cholestane	+91
(1) + (2) − (3)	+74
3α,5α-dihydroxy-5α-cholestane	+69
1. 3α-hydroxy-5α-cholestane	+126
2. 4β hydroxy-5α-cholestane	+113
3. 5α-cholestane	+91
(1) + (2) − (3)	+148
3α,4β-dihydroxy-5α-cholestane	+157
1. 2β-hydroxy-5α,22a-spirostane	−210
2. 3α-hydroxy-5α,22a-spirostane	−242
3. 5α,22a-spirostane	−273
(1) + (2) − (3)	−179
2β,3α-dihydroxy-5α,22a-spirostane	−225
1. 2ξ-bromo-3,17-dioxoandrosta-4-ene	+620
2. 6α-bromo-3,17-dioxoandrosta-4-ene	+394
3. 3,17-dioxoandrosta-4-ene	+566
(1) + (2) − (3)	+448
2ξ,6α-dibromo-3,17-dioxoandrosta-4-ene	+475

substituents on the ring if both are in equatorial positions. Insofar as the six-membered rings in the steroids resemble cyclohexane chairs it is readily seen that the optical rotation of any steroid substituted with axially symmetrical groups X and Y in the cyclohexane rings at positions m and n in the configurations χ or η (where χ and η stand for either endo- or exo-, i.e. for either α or β in the usual terminology employed by steroid chemists) should be related in the following way to the rotations of the monosubstituted and unsubstituted steroids,

$$m\chi\text{X-}n\eta\text{Y-steroid} = m\chi\text{X-steroid} + n\eta\text{Y-steroid} - \text{steroid}$$

provided that the groups X and Y are *both* in either axial positions or in coplanar equatorial positions. This relationship is tested in Table 10, where the groups X- and Y- are in four instances hydroxyl groups, which are assumed to have axial symmetry due to rotation about the C—O bond attaching them to the ring. In one instance X- and Y- are bromine atoms. The predicted relationship, while not obeyed perfectly, is

[65] J. P. Mathieu and A. Petit, *Tables de Constantes et Données Numériques. Constantes Sélectionnées. Pouvoir Rotatoire Naturel I. Stéroides.* Masson et Cie, Paris (1956).

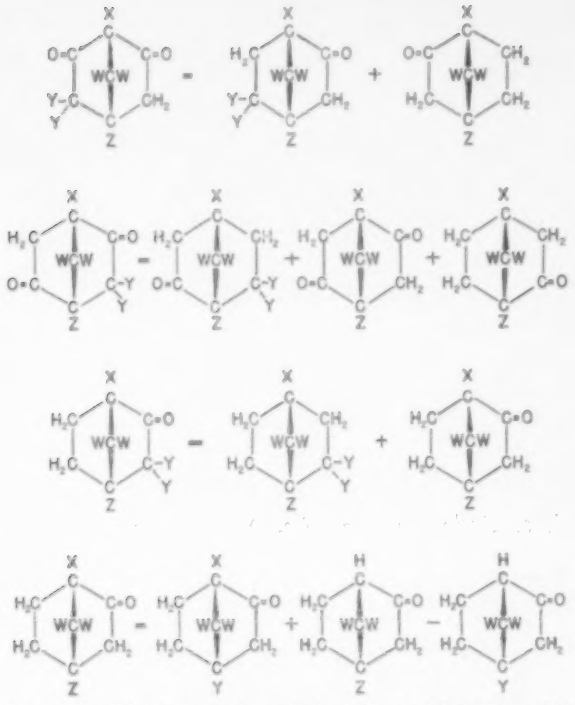


FIG. 19. Further relationships between the optical rotations of derivatives of [1.2.2] bicycloheptane. The symbols W, X, Y and Z denote any axially symmetric groups (H, CH_3, halide, CN, freely orienting OH, etc.).

nevertheless in reasonably good accord with the observations. What discrepancies there are might be accounted for by the failure of the hydroxyl groups to behave as truly axially symmetric groups because of partially restricted rotation in one or more compounds in each set. The presence of the five-membered ring "D" in these steroids might also cause slight distortions of the six-membered rings from the assumed symmetrical cyclohexane chair conformations.

Tetrahedron, 1961, Vol. 13, pp. 106 to 122. Pergamon Press, Inc. Printed in Northern Ireland

SOME APPLICATIONS OF THE CONFORMATIONAL DISSYMMETRY RULE

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THE twisted chain of four atoms (I)[1] is not superposable on its mirror image and so is dissymmetric;[2] as such it might be expected to be, of itself, optically active.[3] This optical activity will, of course, be observable only when enantiomeric conformational units are present in unequal amounts because of restraints on rotation about the central bond. Such restraints can be provided by structure (as when the central bond is part of a ring), by steric hindrance (as in the optically active biphenyls) or by a dissymmetric environment which makes one conformational unit more stable than its enantiomer.[4] The concept that conformational dissymmetry may be an important

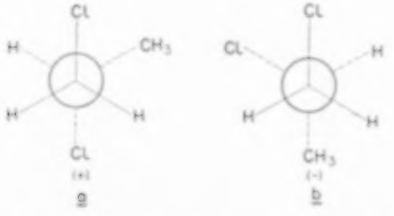
$\Delta[M]_D = +kXY$

I

source of optical activity in organic compounds in general has been explicitly employed in many discussions of optical activity, among which may be mentioned those of Kuhn[5], Kirkwood[6], Eyring[7] and Bernstein and Pedersen[8]. Whiffen[9] reduced this

$$\Delta[M]_D = kXY$$

[1] This simple chain of four atoms will be referred to as a "conformational unit".
[2] G. W. Wheland, *Advanced Organic Chemistry* (2nd Ed.) p. 151. John Wiley, New York (1949). Properly speaking, this unit is asymmetric only when X and Y are different.
[3] See K. Mislow, *Trans. New York Acad. Sci.* **19**, 298 (1957), for references to some of the early expressions of this idea and for references to examples of optically active conformational enantiomers.
[4] We will confine our attention to cases in which the dissymmetric environment is provided by one or more asymmetric atoms within the molecule containing the conformational unit.
[5] W. Kuhn, *Trans. Faraday Soc.* **26**, 293 (1930); comment on p. 352.
[6] J. G. Kirkwood, *J. Chem. Phys.* **5**, 479 (1937); W. W. Wood, W. Fickett and J. G. Kirkwood, *Ibid.* **20**, 561 (1952). In the latter paper the rotatory properties of 1,2-dichloropropane are considered to be controlled



a b

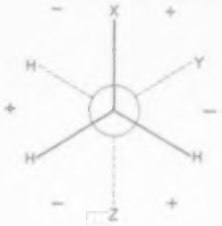
by the relative prevalence of the dextrorotatory conformation *a* and the levorotatory conformation *b* as influenced by solvent properties and temperature.
[7] See W. J. Kauzmann, J. E. Walter and H. Eyring, *Chem. Rev.* **26**, 338 (1940). This review emphasizes the point that the large rotations of many cyclic compounds reflect the dissymmetry of relatively rigid conformations.
[8] H. J. Bernstein and E. E. Pedersen, *J. Chem. Phys.* **17**, 885 (1949).
[9] D. H. Whiffen, *Chem. & Ind.* 964 (1956).

principle to a convenient rule which, in modified form,[10] appears to be rather widely applicable. The modified rule, for which the name: "Conformational Dissymmetry Rule" is proposed, can be stated as follows:

(a) The "conformational unit" I is dextrorotatory[11] in the absolute configuration shown.

(b) The magnitude of this rotatory effect is proportional to constants characteristic of the terminal atoms, X and Y:[12]

(c) The rotatory power of a full conformation (as II) is the sum of the rotatory contributions of the six constituent conformational units.



II

$$[M]_D = k(XY - HY - HX + HH) = k(X - H)(Y - H)$$

X	Y	k(X − H)(Y − H)
Br	CH_3	180°
CH_3	CH_3	60
NH_2	CH_3	55
OH	CH_3	50

II

This conformation will be dextrorotatory when X and Y are both more polarizable than hydrogen (compare conformations *a* and *b*[6]). Numerical values for representative full conformations have been obtained empirically;[10] some of these values are shown in II.

Whiffen[9] showed that his form of this rule could be used to predict the sign and magnitude of rotation of the polyhydroxycyclohexanes and the pyranose sugars and pyranosides. The modified rule,[10] using somewhat different constants, permits similar predictions in the carbohydrate series and has been extended to open-chain, saturated carbocyclic, endocyclic unsaturated and deuterium compounds. In certain cases additional effects resulting from gross asymmetry about a carbon atom or from general molecular dissymmetry must also be taken into account. It is the purpose of this

[10] J. H. Brewster, *J. Amer. Chem. Soc.* **81**, 5475, 5483, 5493 (1959); *Tetrahedron Letters* No. 20, 23 (1959).

[11] This rule is designed for use with rotations measured at the sodium D line. In general, however, a center of optical activity which produces a positive Cotton effect at the wave length of an "optically active absorption band" will produce dextrorotation in longer wave length regions of transparency. Dispersion measurements, even if they produce only plain curves, are therefore recommended as a means of checking up on interpretations made by use of this rule, particularly when a substance may be expected to contain several optically active absorption bands at different wave lengths.

[12] While Whiffen[9] considered this effect to be determined by the terminal atoms, he did not propose this simple proportionality. In consequence, his empirical numerical values could be added and subtracted but not factored; this placed a severe limitation on the extension of his treatment. The empirical numerical values obtained in the present author's treatment roughly follow the expression:

$$\triangle[M]_D = 165R_X^{1/3}R_Y^{1/3}$$

where R_X and R_Y are atomic refractions of the terminal atoms. *A priori*, it seems likely that different constants might be needed when the central atoms differ significantly from carbon in polarizability.

paper to illustrate the mechanics of the application of this rule, a clear understanding of this point being necessary if significant tests and criticisms are to be made. Some of the present limitations of this rule will also be pointed out.

Relatively rigid compounds.

The lupine alkaloids. The application of this rule to rigid saturated compounds is straightforward; each bond is examined in turn and its expected rotatory contribution noted, the sum of these contributions being the predicted rotation. We may illustrate the mechanics of this rule, and some of its limitations, by atttempting to predict the absolute configurations of the lupine alkaloids. It should be emphasized that until the limitations of this rule have been defined all such predictions require independent confirmation; we are then, at best, making only tentative assignments of absolute configuration.

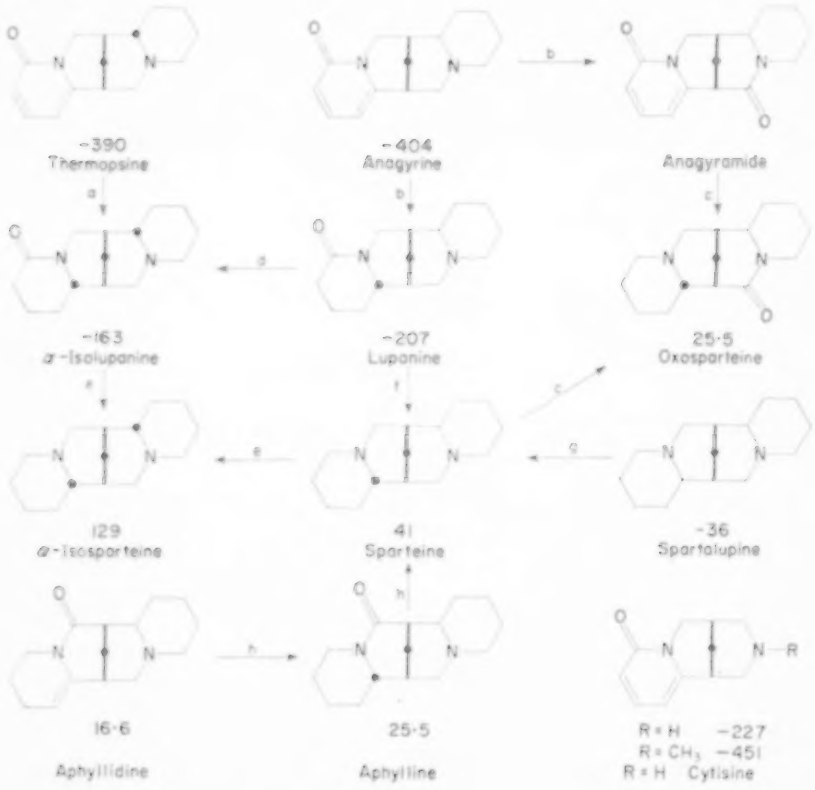
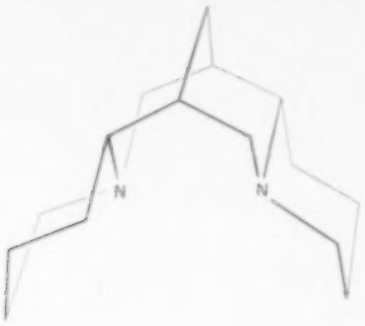


Chart I.

Chart I shows the relative configurations of these alkaloids as deduced by Marion

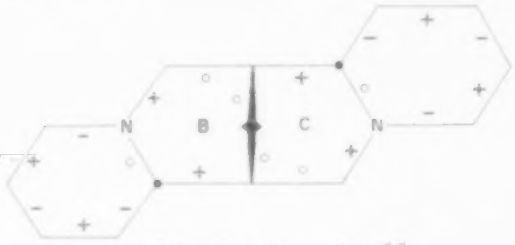
[a] W. F. Cockburn and L. Marion, *Canad. J. Chem.* **29**, 13 (1951)
[b] H. R. Ing, *J. Chem. Soc.* 504 (1933).
[c] F. Galinovsky and E. Stern, *Ber. Dtsch. Chem. Ges.* **76**, 1034 (1943); **77**, 132 (1944).
[d] L. Marion and N. J. Leonard, *Canad. J. Chem.* **29**, 355 (1951).
[e] L. Marion, F. Turcotte and J. Ouellet, *Canad. J. Chem.* **29**, 22 (1951).
[f] G. R. Clemo, R. Raper and C. S. Tenniswood, *J. Chem. Soc.* 429 (1931).
[g] M. Carmack, B. Douglas, E. W. Martin and H. Suss, *J. Amer. Chem. Soc.* **77**, 4435 (1955).
[h] A. Orekhov, *J. Gen. Chem.* (*U.S.S.R.*) **7**, 2048 (1937).

and Leonard[13] and as confirmed by an X-ray diffraction study of α-isosparteine[14](III).



III
α-Isosparteine

The rotatory effect of each of the bonds of the all-chair form of α-isosparteine is shown in Fig. IV; the molecular rotation predicted for this conformation and configuration is 120°, there being a net excess of two dissymmetric conformations of rotatory power $k(C - H)(C - H)$. The close approach of the two nitrogen atoms in this conformation



Ring conformation		Calc $[M]_D$
B	C	
chair	chair	120 (above)
chair	boat	120
boat	chair	120
boat	boat	120

α-Isosparteine

IV

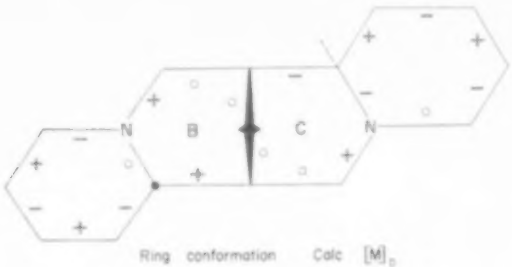
suggests that rings B and C might adopt boat conformations in each of the epimeric sparteines. These conformations can be analyzed if it be assumed that the conformational unit V has the same rotatory power as I (this would be true if the magnitude of

V

[13] L. Marion and N. J. Leonard, *Canad. J. Chem.* **29**, 355 (1951).
[14] M. Przybylska and W. H. Barnes, *Acta Cryst.* **6**, 377 (1953).

the rotatory effect varied with the sine of the angle ϕ). As seen in the tabulation under Fig. IV, all of the conformations of α-isosparteine are predicted to have the same rotation; this value is in reasonable agreement (in magnitude) with rotations reported for the hydrate, $[M]_D$ 129 (abs. alc.),[15] 141 (methanol).[16]

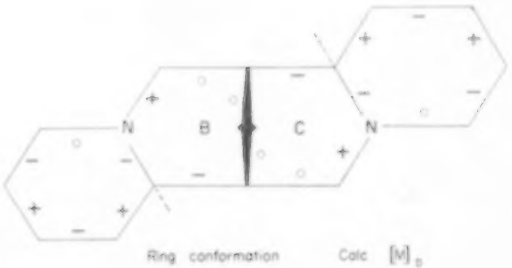
A similar analysis of conformational dissymmetry in sparteine (VI) and spartalupine (VII) indicates that these epimers should have smaller rotations, with sparteine



Ring B	conformation C	Calc	$[M]_D$
chair	chair	0	(above)
chair	boat	60	
boat	chair	0	
boat	boat	60	

Sparteine

VI



Ring B	conformation C	Calc	$[M]_D$
chair	chair	-120	(above)
chair	boat	-60	
boat	chair	-60	
boat	boat	0	

Spartalupine

VII

having the same sign of rotation as α-isosparteine and spartalupine having the opposite sign. The configurationally related alkaloids do, in fact, have rotations of the predicted sign and order of magnitude [sparteine $[M]_D$ 41 (abs. alc.);[15] spartalupine, $[M]_D$ −36 (abs. alc.)][17,18].

This rule is not applicable at present to lactams (as lupanine) or fused ring aromatic compounds (as thermopsine) in part because reliable rotation constants for bonds near highly unsaturated groups are not yet available. Perhaps more important

[15] L. Marion, F. Turcotte and J. Ouellet, *Canad. J. Chem.* **29**, 22 (1951).
[16] N. J. Leonard and R. E. Beyler, *J. Amer. Chem. Soc.* **72**, 1316 (1950).
[17] R. Greenhalgh and L. Marion, *Canad. J. Chem.* **34**, 456 (1956).
[18] M. Carmack, B. Douglas, E. W. Martin, and H. Suss, *J. Amer. Chem. Soc.* **77**, 4435 (1955).

is the likelihood that in these compounds the whole molecule forms a single dissymmetric pattern of polarizability ("permolecular dissymmetry") the rotatory effect of which is dominant. Thus, the alkylation of cytisine (Chart I) produces an enormous rotatory shift which cannot be explained at all by the Conformational Dissymmetry Rule. It is the author's opinion that the approach exemplified by the Octant Rule may be needed to account for such long-range "permolecular" effects in unsaturated compounds; a possible alternative is analysis of such patterns of polarizability as extended dissymmetric conformations.

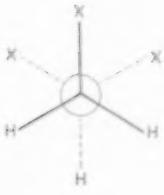
Flexible compounds

An estimate of the relative prevalence of each possible molecular conformation must be made if the Conformational Dissymmetry Rule is to be applied to a flexible compound. The following rules[10] permit a convenient but approximate conformational analysis of open-chain compounds:

(a) Only staggered conformations are "allowed".

(b) The five atom conformation VIII is "prohibited" when the terminal atoms are larger than hydrogen. The strain in this conformation would be equivalent to that found in the corresponding 1,3-diaxial cyclohexane.

VIII



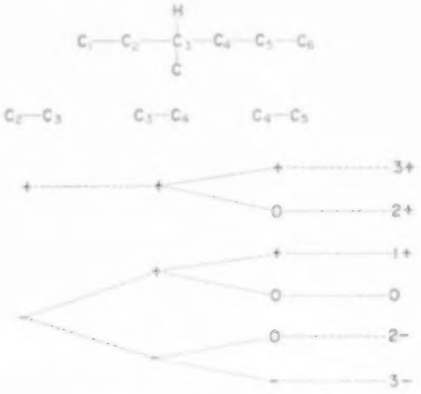
IX

X

XI

(c) The conformation IX is "prohibited". The *cis* conformations of 1,1,2-trichloroethane[19] and of 2-methylbutane[20] are less stable than the corresponding *skew* forms by at least 4 kcal. By the same argument, the two conformations X and XI, which in 1,1,2,2-tetrachloroethane[19] and 2,3-dimethylbutane[20] have almost the same energies, must be "allowed".

(d) All "allowed" conformations are, in the first approximation, equal in energy. This rule is the weakest of the four and, in many cases, can obviously be relaxed; it is really only needed in the conformational analysis of compounds with long flexible chains.



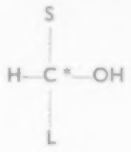
XII

These rules, used systematically, permit a simple, if approximate, conformational analysis of a compound such as (+) 3-methylhexane (XII). Bond C_2—C_3 is "allowed" to have two conformations having rotatory powers of 60° and −60°. When this bond is in the dextrorotatory conformation the only "allowed" conformation of bond C_3—C_4 is also dextrorotatory (rules b and c). When the conformation at C_3—C_4 is dextrorotatory the levorotatory conformation at C_4—C_5 is "prohibited" (rule b), the others being "allowed". (These restraints on rotation at bonds C_3—C_4 and C_4—C_5 are readily seen by use of ball-and-stick models.) For the case when bond C_2—C_3 has its dextrorotatory conformation, there are, then, two molecular conformations having rotatory powers of 180° and 120°. When the conformation at C_2—C_3 is levorotatory the rest of the molecule has greater rotational freedom. Both the dextrorotatory and the levorotatory conformations at C_3—C_4 are "allowed" and for each of these cases two conformations are "allowed" at C_4—C_5. These four molecular conformations have predicted rotations of 60°, 0°, −120° and −180°. This analysis is summarized in a convenient form by the branching diagram shown under XII. Note that if each conformation is considered equally probable the net rotation of the mixture would be 10°; this compares well with the observed value of 9·9°.[21] In effect, the asymmetric atom has provided a dissymmetric environment for the ethyl and propyl groups and prevented

[19] J. R. Thomas and W. D. Gwinn, *J. Amer. Chem. Soc.* **71**, 2785 (1949).
[20] D. W. Scott, J. P. McCullough, K. D. Williamson and G. Waddington, *J. Amer. Chem. Soc.* **73**, 1707 (1951).
[21] R. E. Marker, *J. Amer. Chem. Soc.* **58**, 976 (1936).

them from acting as cylindrically symmetrical substituents. The sign and magnitude of rotation of a large number of simple open-chain compounds can be predicted by this method.[10]

Freudenberg[22] has pointed out that the acid phthalate of a secondary carbinol having the absolute configuration XIII (S is a small alkyl group, L is a large alkyl group) is more dextrorotatory ($[M]_D$) than the parent carbinol. The "Phthalate Rule" can be rationalized by use of the Conformational Dissymmetry Rule; this analysis suggests a more general "Benzoate Rule".



XIII

A benzoate ester will tend to adopt the planar conformation XIV in which resonance effects are maximized and steric interactions between the phenyl group and the alkyl group are minimized. The carbonyl oxygen atom has appreciable steric requirements and will tend to align itself so that it is flanked by the two smallest substituents on the carbinol carbon atom. Thus the benzoate of XIII will tend to assume conformation XV about the bond C*—O (the shaded circle represents the 1,4-axis of the

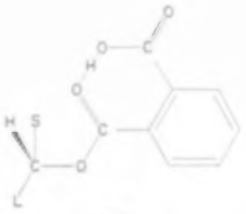
XIV

XV XVI

benzene ring). This conformation, which is, of course, not found in the parent carbinol, should be strongly dextrorotatory since the alkyl group, S, will be more polarizable than the hydrogen atom. If the steric requirements of L and S are grossly different, conformation XV will be predominant; as the steric requirements of the two groups, L and S, become more nearly alike, more of the levorotatory conformation XVI will be present. Accordingly, if L and S are similar in polarizability, the magnitude of the rotation difference between the benzoate and the carbinol ($\Delta = [M]_{benzoate} - [M]_{carbinol}$) will be related to the difference in the steric requirements of the two groups. This will not, however, be a simple relationship since benzoylation will alter the conformational freedom of L and S and so produce additional (but probably small)

[22] K. Freudenberg, *Stereochemie* p. 696. Deuticke, Leipzig (1933).

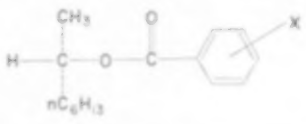
conformational dissymmetry effects. This analysis indicates that similar rotation shifts should be observed when the benzoate group carries *m*- or *p*- substituents. Acid phthalates might be expected to follow this rule because they would probably tend to have the hydrogen-bonded structure XVII, which would reinforce the required rigidity of the benzoyl group. It would not be surprising if other *o*-substituents produced anomalous rotation shifts. This rule would be expected to apply equally well to open-chain and cyclic saturated carbinols.



XVII

As seen in Tables 1 and 2, *m*- and *p*- substituents do not interfere with the operation of this rule; electron-withdrawing *o*-substituents increase the magnitude of the rotatory shifts. There is a regular variation in rotatory shift as a function of the relative steric requirements of the two alkyl groups (Table 3). This effect is also seen in the 2-methylcyclohexanols (XVIII), the borneols (XIX), the cholestanols (XX), the menthols (XXI) and the carvomenthols (XXII). In the last two series the shifts are not always as large as might be expected, perhaps because of conformational mobility of the ring. These regularities suggest that configurational assignments might be based on the occurrence of large rotation shifts of this nature.

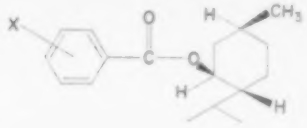
TABLE 1. MOLECULAR ROTATIONS OF BENZOATES OF (+) 2-OCTANOL



X	Temperature	*o*-	*m*-	*p*-
NO_2	20	122[a]	108[a]	
	30			118[a]
	90	115[a]	97[a]	109[a]
OCH_3	20	33[a]	94[a]	113[a]
	90	45[a]	90[a]	109[a]
CO_2H		134 (alc.)[b]		
		119 (chf.)[b]		
	Benzoate		78[c]	

[a] H. G. Rule and A. H. Numbers, *J. Chem. Soc.* 2116 (1926).
[b] R. H. Pickard and J. Kenyon, *J. Chem. Soc.* **99**, 45 (1911).
[c] J. Kenyon and R. H. Pickard, *J. Chem. Soc.* **107**, 115 (1915).
All rotations positive. Unless otherwise noted all esters in homogeneous state.

TABLE 2. MOLECULAR ROTATIONS OF BENZOATES OF (+) MENTHOL



X	Temperature	*o*-	*m*-	*p*-
NO_2	65	381[a]	250[a]	235[a]
CO_2H	20	332[b]		259[c]
CO_2CH_3	20	265[b]		246[c]
I	20	246[d]	235[d]	241[d]
CH_3	20	231[e]	240[e]	246[e]
Br	20	205[f]	239[f]	239[f]
Cl	20	195[g]	237[g]	237[g]
OCH_3	20	145[e]	247[e]	239[e]
$N(CH_3)_2$	20	206[h] (alc.)		258[e]

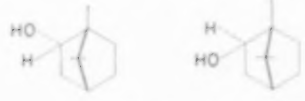
Benzoate	236[a]
α-Naphthoate	246[i] (alc.)
β-Naphthoate	283[i] (benz.)

[a] J. B. Cohen and H. P. Ames, *J. Chem. Soc.* **87**, 1190 (1905).
[b] J. B. Cohen, D. Woodroffe and L. Anderson, *J. Chem. Soc.* **109**, 222 (1916).
[c] J. B. Cohen and H. S. de Pennington, *J. Chem. Soc.* **113**, 57 (1918).
[d] J. B. Cohen and H. S. Raper, *J. Chem. Soc.* **85**, 1271 (1904).
[e] J. B. Cohen and H. W. Dudley, *J. Chem. Soc.* **97**, 1732 (1910).
[f] L. Tschugaeff, *J. Russ. Phys. Chem. Soc.* **34**, 606 (1902).
[g] J. B. Cohen and S. H. C. Briggs, *J. Chem. Soc.* **83**, 1213 (1903).
[h] H. G. Rule and W. E. MacGillivray, *J. Chem. Soc.* 401 (1929).
[i] H. Rupe and M. Silberberg, *Liebigs Ann.* **327**, 193 (1903).

Unless otherwise noted all rotations on fused (supercooled) esters; for the most part, these values are not strongly influenced by temperature changes or solvent effects. All of this work was actually done with esters of (−) menthol; the benzoates all have the same sign of rotation as the parent alcohol.

[M]	42 (homog)[23]	15 (homog)[23]
Acid phthalate	166 (benz.)[23]	101 (benz.)[23]
Δ	124	86

XVIII

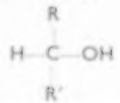


[M]	58 (toluene)[24]	−33 (toluene)[24]
Acid phthalate	171 (alc)[25]	−234 (alc)[25]

XIX

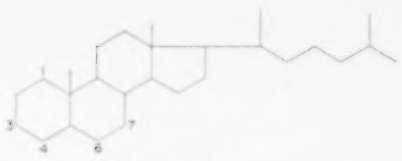
[23] G. A. C. Gough, H. Hunter and J. Kenyon, *J. Chem. Soc.* 2052 (1926).
[24] A. Haller, *Ann. Chim.*, VI, **27**, 392 (1892).
[25] R. H. Pickard and W. O. Littlebury, *J. Chem. Soc.* **91**, 1973 (1907).

TABLE 3. MOLECULAR ROTATIONS OF SATURATED SECONDARY ACYCLIC CARBINOLS AND THEIR ACID PHTHALATES



R′	R—CH_3[a]			R—C_2H_5[b]			R—$CH(CH_3)_2$[c]			R—$C(CH_3)_3$[d]		
	Carbinol	Acid phthalate	Δ	Carbinol	Acid phthalate	Δ	Carbinol	Acid phthalate	Δ	Carbinol	Acid phthalate	Δ
CH_3	0	0		−10·3	−74·5	−64·2	−4·3	−89·3	−85	−7·8	−160	−152
CH_2CH_3	10·3	74·5	64·2	0	0		15·4	−1·2	−16·6	39	3·3	−36
$(CH_2)_2CH_3$	12·1	87·2	75·1	2·01	10·45	8·4	24·7	20·9	−3·8	55	−8·4	−63
$(CH_2)_3CH_3$	11·8	108·5	96·7	9·4	44	35	33·3	38·7	5·4	60	13	−47
$(CH_2)_4CH_3$	12·0	116	104	10·7	59·7	49	32·9	39·3	6·4			
$(CH_2)_5CH_3$	12·7	120·3	108	11·6	41·2	29·6	33·9	36·1	2·2			
$(CH_2)_6CH_3$	12·9	120·1	107	10·6	38·8	28·2						
$(CH_2)_7CH_3$	13·7	119·3	106	10·7	38·1	27·4	34·5	56·3	21·8			
$(CH_2)_8CH_3$	14·0	119·5	106	11·1	39·5	28·4						
$(CH_2)_9CH_3$	14·5	121·0	107	12·4	44·7	32·3	34·5	50·3	15·8			
$CH(CH_3)_2$	4·3[e]	89·5[e]	85	−15·4[e]	1·2[e]	16·6	0	0		14	0	−14
$C(CH_3)_3$	7·8[f]	160[f]	152	−39[f]	−3·3[f]	36	−14[f]	0[f]	14	0	0	

[a] R. H. Pickard and J. Kenyon, *J. Chem. Soc.* **99,** 45 (1911).
[b] *Idem., J. Chem. Soc.* **103,** 1923 (1913).
[c] Idem., *J. Chem. Soc.* **101,** 620 (1912).
[d] W. M. Foley, F. J. Welch, E. M. LaCombe and H. S. Mosher, *J. Amer. Chem. Soc.* **81,** 2779 (1959).
All rotations positive unless otherwise noted. Carbinols homogeneous; acid phthalates in chloroform



$[M]_D$[26]

	α—OH			β—OH		
Position	Carbinol	Benzoate	Δ	Carbinol	Benzoate	Δ
1				76	1	−75
3	97	114	17	90	98	8
4	12	−98	−110	113	280	167
6	136	307	171			
7	43	−108	−151	202	428	226

XX

$[M]_D$[27]

	a	Δ	b	Δ	c	Δ	d	Δ
OH	−77		32		−40		−3·5	
PNB	−232	−155	55	23	−76	−36	16	19·5
DNB	−278	−201	84	52	−95	−53	33	36·5

OH, carbinol; PNB, *p*-nitrobenzoate; DNB, 3,5-dinitrobenzoate.

XXI

$[M]_D$[28]

	a	Δ	b	Δ	c	Δ	d	Δ
OH	−43		65		28		54	
PNB	−158	−115	69·5	4·5	83	55	72·5	18·5*
DNB	−185	−142	79	14	94	66	56	2*
APh	−176	−133	148	83				

OH, carbinol; PNB, *p*-nitrobenzoate; DNB, 3,5-dinitrobenzoate; APh, acid phthalate.

* The purity of this isomer, neoisocarvomenthol, is uncertain; these shifts are in a direction opposite to that predicted.

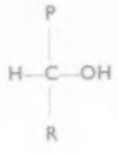
XXII

[26] Data cited by J. P. Mathieu and A. Petit in *Tables de Constantes et Donnees Numeriques. 6. Constantes Selectionees. Pouvoir Rotatoire Naturel. I. Steroides.* Masson, Paris (1956).

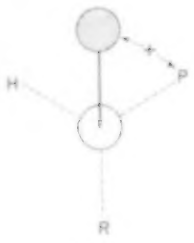
[27] J. Read, W. J. Grubb and D. Malcolm, *J. Chem. Soc.* 170 (1933); J. Read and W. J. Grubb, *J. Chem. Soc.* 313 (1934).

[28] R. G. Johnston and J. Read, *J. Chem. Soc.* 1138 (1935).

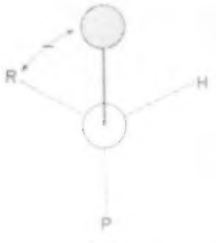
A parallel analysis indicates that when the two substituents at the carbinol center have similar steric requirements but differ in polarizability then the carbinol having configuration XXIII (P is the more polarizable group, R is the less polarizable group) will show a dextrorotatory shift (compare XXIV and XXV). Mills[29] has already pointed out that such shifts do indeed occur in the case of allylic alcohols of the terpene and steroid series; the example shown in XXVI is typical.



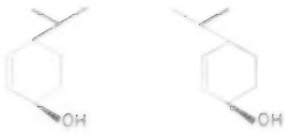
XXIII



XXIV



XXV



$[M]_D$

	a	Δ	b	Δ
OH	104		165	
PNB	540	436	494	329
DNB	518	404	500	335

XXVI

This rule cannot yet be safely applied to the phenyl alkyl carbinol series where the highly polarizable phenyl group is usually also the most bulky substituent. Very large solvent effects are noted in the rotations of the acid phthalates of this series.

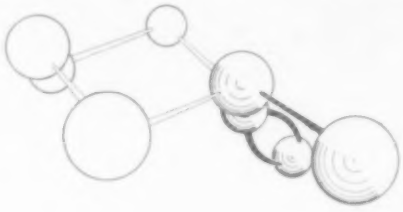
Cyclic ketones

We are attempting to establish the limitations of the Conformational Dissymmetry Rule by testing its applicability to groups of conformationally simple compounds of known configuration. The thorough work of Djerassi *et al.*[31] has made it possible

[29] J. A. Mills, *J. Chem. Soc.* 4976 (1952).
[30] D. T. C. Gillespie, A. K. Macbeth and J. A. Mills, *J. Chem. Soc.* 996 (1948).
[31] Reviewed in C. Djerassi, *Optical Rotatory Dispersion.* McGraw-Hill, New York (1960).

(indeed, necessary) to try out this rule on cyclic ketones. For the most part it gives predictions equivalent[32] to those made by use of the Octant Rule.[33] The cases in which the two rules give opposite predictions are of especial interest as they indicate an area where experimental testing is needed. Although the tests we propose have not yet been made (Jan. 1960) it seems desirable to bring this discrepancy into the open since it indicates a limitation of one or the other of the rules.

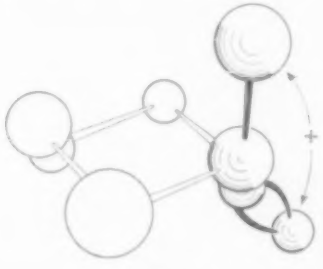
An equatorial substituent at position 2 of a cyclohexanone is almost coplanar with the carbonyl group (see the shaded portions of XXVII); this nearly symmetrical con-



XXVII

Ball-stick model of an equatorial 2-substituted cyclohexane. The shaded balls show the nearly symmetrical conformation formed by the equatorial substituent, two ring carbon atoms and the carbonyl oxygen atom.

formation would be expected to produce little rotatory effect. An axial substituent at the same position forms a highly dissymmetric conformation with the whole carbonyl group; this particular conformation and configuration has the same geometry as V (let X be the axial substituent and Y the carbonyl oxygen atom) and so should produce a positive Cotton effect at some absorption band of the carbonyl group.[32] This will, of course, only be observable if the axial substituent at position 6 (which is in an enantiomeric conformation) has a lower polarizability. It is to be expected, then, that while alkyl, chloro and bromo groups in the configuration shown in XXVIII will produce



XXVIII

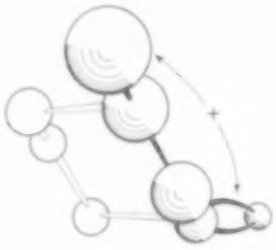
Ball-stick model of an axial 2-substituted cyclohexanone. The shaded balls show the highly dissymmetric conformation formed by the axial substituent, two ring carbon atoms and the carbonyl oxygen atom.

[32] This equivalence is rather rough. The Conformational Dissymmetry Rule does not take into account the geometry of electronic transitions and so cannot be used to predict which absorption bands will be optically active. It can only be said that a dextrorotatory effect in the visible will be related to a positive Cotton effect at the wave length corresponding to *some* absorption band of a chromophore that forms a part of a dissymmetric conformation.

[33] See ref. 31, pp. 178–190.

positive Cotton effects, a fluorine atom (less polarizable than hydrogen) will produce a negative Cotton effect; such results have been observed.[34] Thus the two rules appear to give concordant predictions for equatorial and axial substituents at the 2 position.

An equatorial substituent at the 3 position lies in a plane containing the carbonyl carbon atom (position 1) and the carbon atoms at positions 2 and 3. While this plane is *per se*, symmetrical, it is skewed relative to the double bond of the carbonyl group (see the shaded portions of XXIX). The substituent, then extends the dissymmetric conformation about the bond C_1—C_2 and might be expected to have some rotatory effect;



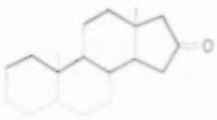
XXIX

Ball-stick model of an equatorial 3-substituted cyclohexanone. The shaded balls show the extended dissymmetric conformation formed by the equatorial substituent, three ring carbon atoms and the carbonyl oxygen atom. The equatorial substituent and the three ring atoms lie in a plane.

this effect would probably be relatively small in the visible since the substituent is at some distance from the carbonyl group but its importance would increase as the optically active absorption band was approached. The direction of this effect, as predicted from XXIX, is in basic accord with the Octant Rule. Further extensions of this conformation should produce larger rotatory effects. Several steroid ketones contain long extended conformations of this sort (see the heavy lines in XXX and XXXI): these

Positive cotton effect

XXX

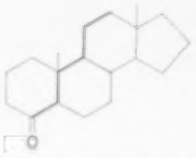


Negative cotton effect

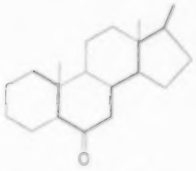
XXXI

[34] C. Djerassi and W. Klyne, *J. Amer. Chem. Soc.* **79**, 1506 (1957); C. Djerassi, J. Osiecki, R. Riniker and B. Riniker, *Ibid.* **80**, 1216 (1958).

ketones show large Cotton effects of the predicted sign.[35] Figures XXXII and XXXIII show that, in this respect, the 4- and 6-ketosteroids have similar dissymmetric conformations and so should give similar negative Cotton effect curves; this is observed.[35] These predictions are similar to those made by use of the Octant Rule.



Negative cotton effect
XXXII



Negative cotton effect
XXXIII

The two rules give different predictions when it comes to axial 3-substituted cyclohexanones. As seen in XXXVI an axial substituent makes a dissymmetric conformation with the carbonyl *carbon atom;* the carbonyl double bond extends this conformation but is not an integral part of it. The relationship between the geometry of the conformation and of the electronic transition is, therefore, different here and we cannot safely predict at which absorption band this conformation will produce its Cotton effect.[32] It is, however, a matter of some interest that if this conformation were to produce a positive Cotton effect at about 300 mμ then simple explanations would be provided for the dispersion curves shown by (+) isomenthone (XXXV; XXVI) and (+) 3-methylcyclopentanone (XXXVII). Under the Octant Rule, the positive Cotton effect of (+) isomenthone is explained[33] by assuming that the conformer XXXVI, in which the isopropyl group is axial, has the dominant rotatory effect (suggesting that XXXVI is present in larger amount than XXXV). Under the other rule, both conformers would be expected to show positive Cotton effects. In the case of (+) 3-methylcyclopentanone (XXXVII), which shows a large positive Cotton effect, the substituent itself, under the Octant Rule, would be expected to produce a negative Cotton effect. It becomes necessary, then, to assume that the substituent favors a particular mode of ring-puckering and that it is this twisted ring structure which produces the large Cotton effect. Under the Conformational Dissymmetry Rule the substituent has the proper orientation for producing a positive Cotton effect[32] even if all puckered forms of the ring were equally probable (in which case the time-average conformation of the ring would be effectively planar, as would the bulk-average conformation).

The Octant Rule interpretations of the dispersion curves of these two compounds are not unreasonable and may well be correct; the author is not aware of published

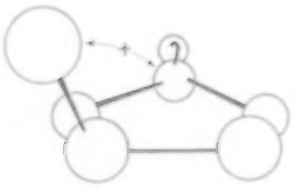
[35] Ref. 31, pp. 42–49.

XXXIV

Ball-stick model of an axial 3-substituted cyclohexanone. The shaded balls show the dissymmetric conformation formed by the axial substituent and three ring atoms (including the carbonyl carbon atom).

XXXV

XXXVI



XXXVII

Ball-stick model of the planar conformation of a 3-substituted cyclopentane. This should approximate to the time-average conformation if ring puckering is not influenced by the presence of the substituent.

data which unequivocally establish this point. The alternative explanations, using the Conformational Dissymmetry Rule, seem simpler but must be regarded with caution because, in the relevant conformations, the carbonyl group plays a role different from that it does in the other cases. It is, thus, possible that the predicted positive rotatory effect of XXXIV is in fact associated with a shorter wave length absorption band of the carbonyl group.[32] These are, clearly, points to be settled by experiment rather than words. If this discussion, by calling attention to this disagreement, leads to the finding of facts which define the limitations of these two rules it will have served its purpose.

Note added in proof (*Jan.* 1961): Preliminary results of our tests of this point indicate that the Octant Rule does, indeed, give correct predictions of the sign of the rotatory effects of axial β-alkyl groups.

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HELICAL SENSE OF POLYPEPTIDES AND PROTEINS

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IN this paper no attempt will be made to give a general review of the rotatory properties of polypeptides and proteins, since such an account has appeared recently.[1] Instead, some work will be summarized, most of it as yet unpublished, which relates to the determination of one aspect of the detailed macromolecular conformations of such molecules. It is now evident, as a result of recent investigations,[2] that the optical rotatory dispersion serves to distinguish the helical conformation of a synthetic polypeptide from random conformations. Since the helical conformation[3] (Fig. 1) appears to be a basic structural unit not only of synthetic polypeptides but also of proteins[4] it is important to be able to determine the sense of helix. Helices are dissymmetric and as such can be either right-handed or left-handed. The work reported here describes two methods (and some results) for determining the relative sense of helical polypeptides through the use of optical rotatory dispersion measurements and has been carried out in most part by Norland, Stryer and Fasman in our laboratory.

Although the equation (1) proposed by Moffitt[5] now is recognized

$$[\alpha]_\lambda = \left(\frac{100}{M_0}\frac{\eta^2+2}{3}\right)\left[a_0\frac{\lambda_0^2}{\lambda^2-\lambda_0^2}+b_0\frac{\lambda_0^4}{(\lambda^2-\lambda_0^2)^2}\right] \qquad (1)$$

to be without sound theoretical basis,[6] it does describe the optical rotatory dispersion of a number of helical polypeptides. For example, poly-γ-benzyl-L-glutamate has been shown to obey equation (1) from 265 to 600 mμ and to yield b_0 values of *minus* 660°.[7] We have extended these measurements below the absorption band due to the benzyl group and find the equation is still fitted down to 240 mμ.[8] It was therefore with some surprise that we observed several years ago that poly-β-benzyl-L-aspartate showed rotatory dispersive properties which fitted the Moffit equation but which gave b_0 values of *plus* 630°.[9] Since polybenzyl-aspartate can be considered as a lower homolog of polybenzyl-glutamate we were unprepared to observe any such radical change in the rotatory dispersive properties. However, these results have been confirmed by repeated

[1] C. Djerassi, *Optical Rotatory Dispersion* Chap. 17 by E. R. Blout, p. 238. McGraw-Hill, New York (1960).
[2] W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci.* **42**, 596 (1956); J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957).
[3] L. Pauling and R. B. Corey, *J. Amer. Chem. Soc.* **72**, 5349 (1950).
[4] C. Cohen and A. Szent-Gyorgyi, *J. Amer. Chem. Soc.* **79**, 248 (1957); J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957); J. A. Schellman, *Compt. Rend. Trav. Lab. Carlsberg, Ser. Chim.* **30**, Nos. 21–26 (1958); G. C. Schellman and J. A. Schellman, *Ibid.* **30**, No. 27 (1958); B. Jirgensons, *Arch. Biochem. Biophys.* **74**, 57, 70 (1958).
[5] W. Moffitt, *J. Chem. Phys.* **25**, 467 (1956); *Idem., Proc. Natl. Acad. Sci.* **42**, 736 (1956).
[6] W. Moffitt, D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci.* **43**, 723 (1957).
[7] P. Doty, *Proceedings of the IVth International Congress of Biochemistry, Vienna* Vol. IX, p. 8. Pergamon Press, London (1959).
[8] R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).
[9] E. R. Blout and R. H. Karlson, *J. Amer. Chem. Soc.* **80**, 1259 (1958); E. R. Blout, *Proceedings of the IVth International Congress of Biochemistry, Vienna* Vol. IX, p. 37. Pergamon Press, London (1959).

syntheses and by hydrolysis of the polymers to the resulting L-amino acids to assure that no racemization had taken place during the synthesis.[8]

The observation that poly-β-benzyl-L-aspartate had a b_0 value equal in magnitude but opposite in sign to that of poly-γ-benzyl-L-glutamate immediately suggested the possibility that the aspartate polypeptide preferred to form the helix of opposite sense to that of the glutamate, but alternative explanations could account for these changes

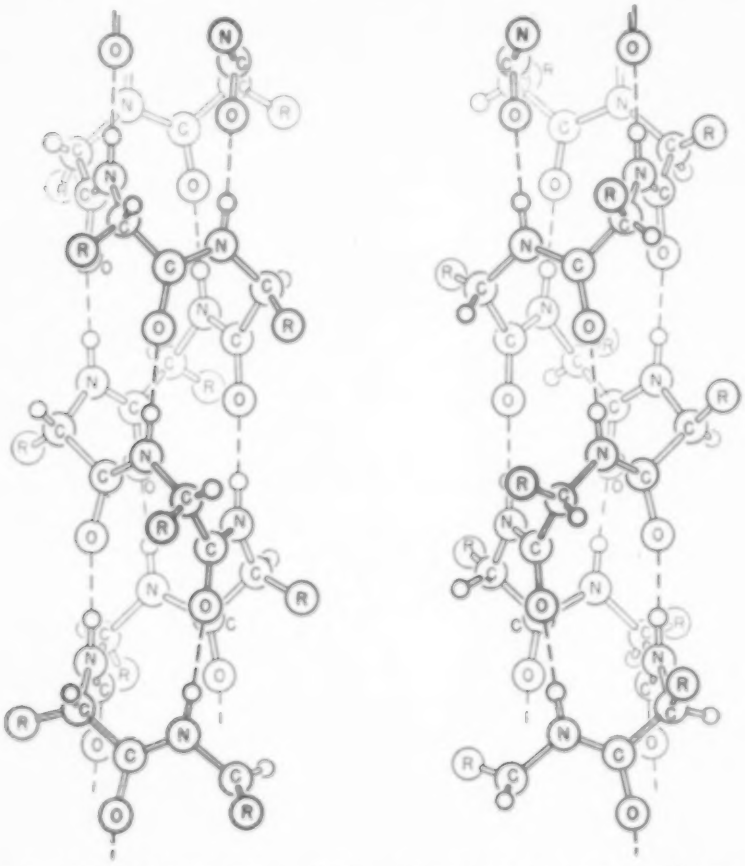


FIG. 1. A drawing showing two possible forms of the α helix; the one on the left is a left-handed helix, the one on the right is a right-handed helix. In both, the amino-acid residues have the L configuration. [Reproduced by permission of the Istituto Lombardo, Milano, from R. B. Corey and L. Pauling, *Rend. ist. lombardo sci.* P 1, **89**, 10 (1955).]

in properties. The problem we set ourselves was to determine the relative sense of helix of polybenzyl-L-glutamate and polybenzyl-L-aspartate. This we believe we have done by studying the optical properties of a series of copolymers of benzyl-L-aspartate with benzyl-L-glutamate and a series of copolymers of benzyl-D-aspartate with benzyl-L-glutamate.

If the sense of helix of poly-γ-benzyl-L-glutamate is opposite to that of poly-β-benzyl-L-aspartate then copolymers of D-aspartate with L-glutamate should show a regular progression of optical properties whereas those composed of L-aspartate and

L-glutamate residues should show a nonregular progression of optical properties because of the change of sense of helix at some point in the copolymer series. Fig. 2 shows the specific rotations of these two series of copolymers in a solvent (chloroform) which favors the helical conformation. It is apparent that the optical rotation in the copolymer series L-benzyl-aspartate:L-benzyl glutamate is non-linear and markedly

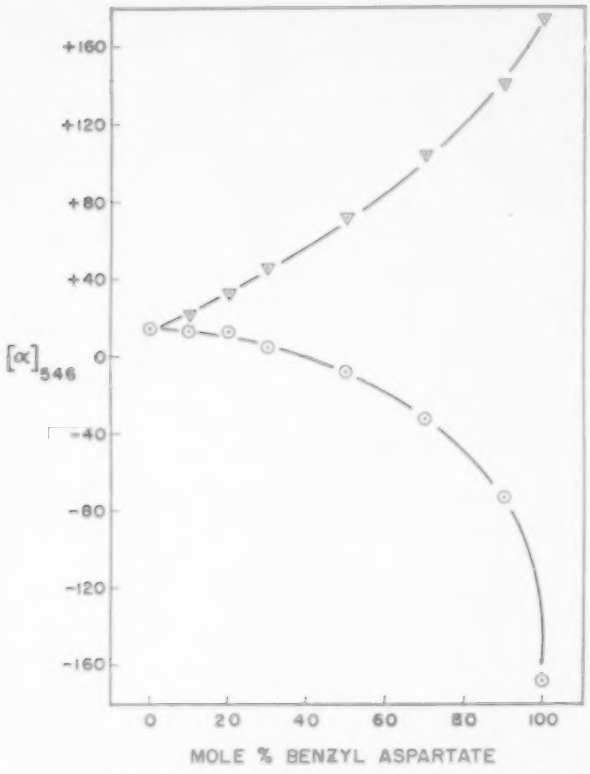


FIG. 2. The specific rotation, $[\alpha]_{546}$, of co-polymers of β-benzyl-L-aspartate:γ-benzyl-L-glutamate ○—○—○, and of co-polymers of β-benzyl-D-aspartate:γ-benzyl-L-glutamate ▽—▽—▽ in chloroform solutons. [Reproduced by permission of the American Chemical Society from R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).]

dependent on the mole percent benzyl-aspartate in the copolymers. On the other hand in the copolymer series D-benzyl-aspartate:L-benzyl-glutamate the optical rotation varies in a regular manner with the mole percent benzyl aspartate.

When the same copolymers are dissolved in a strongly hydrogen-bonding solvent, which destroys the helical conformation, both series of copolymers showed linear variation of $[\alpha]_{546}$ with the mole percent benzyl-aspartate as seen in Fig. 3.

When the optical rotatory dispersions of the copolymers in the helical conformation (chloroform solution) are fitted to equation (1) the coefficient of the second term, b_0, is found to vary in the manner shown by the optical rotation, i.e., there is marked dependence upon mole percent benzyl-aspartate in the L-benzyl-aspartate:L-benzyl-glutamate copolymer series and a relatively linear dependence in the D-benzyl-aspartate:

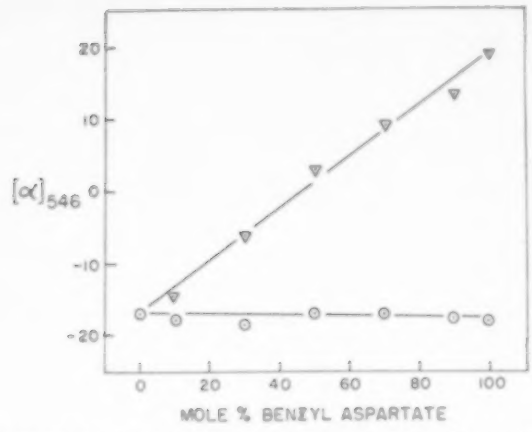


FIG. 3. The specific rotation, $[\alpha]_{546}$, of co-polymers of β-benzyl-L-aspartate:γ-benzyl-L-glutamate ⊙—⊙—⊙, and of co-polymers of β-benzyl-D-aspartate:γ-benzyl-L-glutamate ▽—▽—▽ in dichloroacetic acid solutions. [Reproduced by permission of the American Chemical Society from R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).]

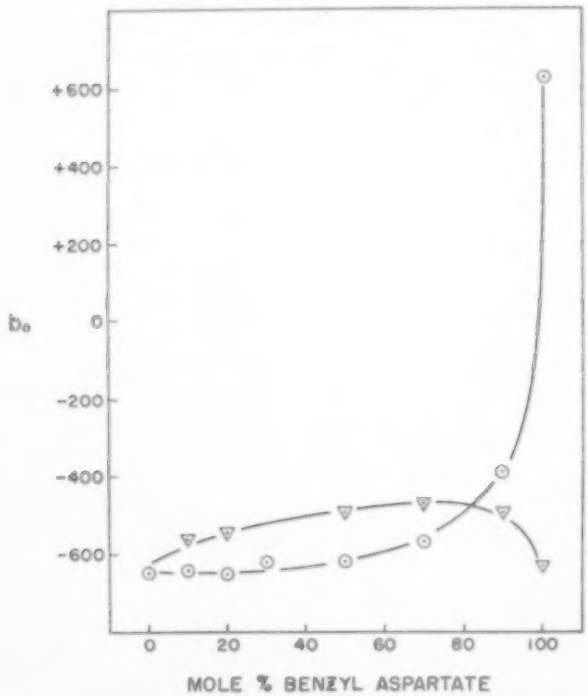


FIG. 4. The b_0 values for the wave length range 365 to 578 mμ (from equation (1) assuming λ_0 = 212 mμ) of co-polymers of β-benzyl-L-aspartate:γ-benzyl-L-glutamate ⊙—⊙—⊙, and co-polymers of β-benzyl-D-aspartate:γ-benzyl-L-glutamate ▽—▽—▽ in chloroform solutions. [Reproduced by permission of the American Chemical Society from R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).]

L-benzyl-glutamate series. These data are shown in Fig. 4. From previous studies on copolypeptides of D- and L-amino acids,[10–12] it might be argued that if L-polybenzyl-aspartate and L-polybenzl-glutamate exist as helices of the opposite sense of twist, the optical rotation and b_0 of *random* copolymers of these polypeptides should change markedly over a relatively narrow range of composition representing a region of transition from one sense of helix to the other. Furthermore, since it has been demonstrated through titration[8] and deuterium exchange[13] studies that the L-polybenzyl-aspartate helix is less stable than the L-polybenzyl-glutamate helix the transition should occur at less than 50 mole percent L-benzyl-glutamate. On the other hand the optical rotation and b_0 of D-benzyl-aspartate:L-benzyl-glutamate copolymers in a helical conformation should show a regular dependence on composition which would be evidence that no change of helix sense occurs in this series. As can be seen from the figures, this is indeed the case and therefore we conclude that there is a change of helical sense in the L:L copolymer series while no change of sense or twist occurs in the D:L copolymer series.

Further evidence for the thesis that the opposite sense of twist exists in L-polybenzyl aspartate than does in L-polybenzyl glutamate is obtained from ultraviolet rotatory dispersion data.[8] The data for two polypeptides over the wavelength range 240–340 mμ are shown in Fig. 5. The dispersion of L-polybenzyl aspartate is anomalous while that of L-polybenzyl glutamate in this region is not anomalous but is complex.

When the optical rotatory dispersion data from 600 to 240 mμ for these polymers was plotted in the manner suggested by Moffitt it was found (as mentioned above and as can be seen in Fig. 6) that polybenzyl-L-glutamate fits the Moffitt equation to yield a b_0 value of *minus* 660°. Polybenzyl-L-aspartate fits the Moffitt equation to wavelengths as short as 265 mμ with a b_0 approximately equal to *plus* 700°, but at shorter wavelengths deviates markedly. It has been suggested that this deviation is due to the onset of a positive Cotton effect which could arise from an interaction between the asymmetric α-carbon atom and the ester chromophore which absorbs in the region 220–230 mμ. The suggestion is supported by the fact that the specific rotation at 240 mμ of polybenzyl-L-aspartate is much more positive than that of polybenzyl-L-glutamate is negative. If it is assumed that the rotation of polybenzyl-L-glutamate is due only to the peptide helix and the residue contributions (and this seems a reasonable assumption since no anomaly is observed) then the increased dextrorotation and the anomaly observed in polybenzyl-L-aspartate around 240 mμ must arise from the approach to another absorption band, presumably that of the ester group. The existence of a Cotton effect would imply that the rotation of the ester group in polybenzyl-L-aspartate is restricted whereas where no Cotton effect is observed, as in polybenzyl-glutamate, the ester group (located here on the γ-carbon) would have not have restricted rotation. Indeed it would be desirable to be able to measure to wave-lengths shorter than 240 mμ in order to settle this point. It must be emphasized however that although the ester groups in polybenzyl-L-aspartate may have restricted rotation, which in turn causes the complex rotation below 265 mμ, the presence of oriented

[10] P. Doty and R. D. Lundberg, *Proc. Natl. Acad. Sci.* **43**, 213 (1957).
[11] A. R. Downie, A. Elliott, W. E. Hanby and B. R. Malcolm, *Proc. Roy. Soc.* A **242**, 325 (1957).
[12] E. R. Blout, P. Doty and J. T. Yang, *J. Amer. Chem. Soc.* **79**, 749 (1957).
[13] E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, W. E. Hanby and T. R. R. McDonald, *Nature, Lond.* **183**, 1736 (1959).

chromophores in the side chain cannot explain all the observed optical effects. To explain the rotatory effects noted above it is necessary to invoke a change of helical sense in L-polybenzyl-aspartate compared to L-polybenzyl-glutamate.

This argument is supported by ultraviolet rotatory measurements on copolymers of D-aspartate and L-aspartate with L-glutamate and some data are shown in Figure 7.

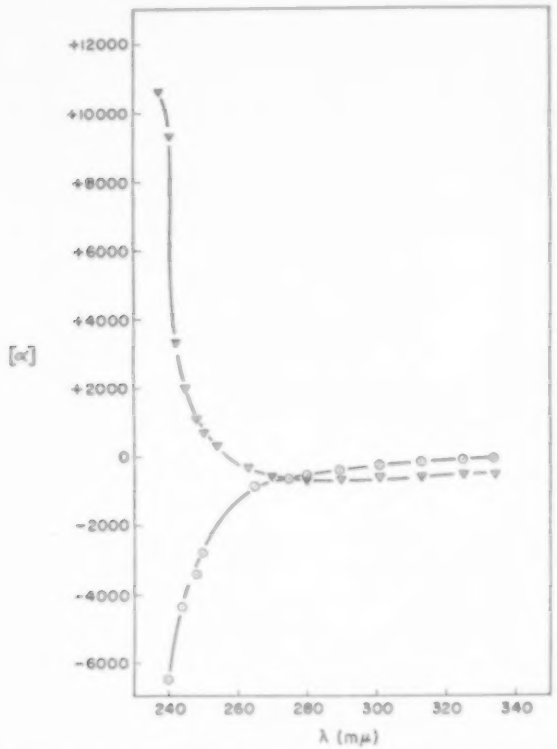


FIG. 5. The ultraviolet rotatory dispersions of poly-β-benzyl-L-aspartate ▽—▽—▽ and poly-γ-benzyl-L-glutamate in chloroform solutions. The specific rotations, $[\alpha]_\lambda$, are plotted as a function of wave length in $m\mu$. [Reproduced by permission of the American Chemical Society from R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).]

The anomaly observed and noted above for L-polybenzyl-aspartate is completely lost upon incorporation of 10 mole percent L-polybenzyl glutamate whereas the incorporation of 10 mole percent L-benzyl-glutamate in D-polybenzyl-aspartate does not result in the loss of the anomaly observed with D-polybenzyl-aspartate. In other words it can be concluded from these data that the incorporation of 10 mole percent L-benzyl glutamate in D-polybenzyl-aspartate does not affect the helical sense whereas the incorporation of 10 mole percent L-benzyl-glutamate in L-polybenzyl-aspartate involves a structural change—that is a change in the sense of the helix. Thus these ultraviolet rotatory dispersions offer additional support for the conclusion that L-polybenzyl-aspartate and L-polybenzyl glutamate exist as helices of the opposite sense.

The fact that polybenzyl-L-aspartate may exist with a sense of helix opposite to that observed with poly-γ-benzyl-L-glutamate is not only interesting in itself but may have

some important implications for protein structures. Since our knowledge of helix content and sense of helix of proteins is meagre it is obvious that all methods should be used which may aid in these structural problems. It is possible that the direct observation of the optical rotatory dispersive properties of proteins is such a tool, but from the results now available[1,2,4] it does not appear that the method has sufficient precision to

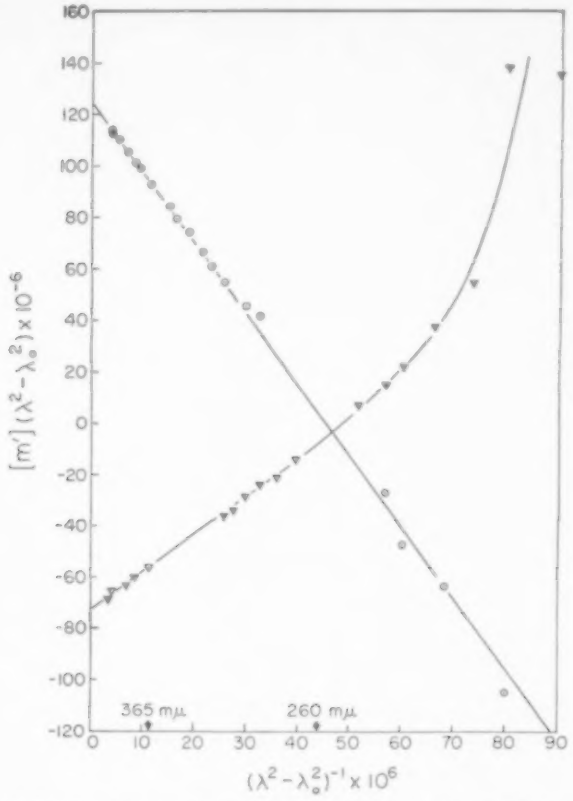


FIG. 6. Moffitt plot (from equation (1) using $\lambda_o = 212$ mμ) of the optical rotatory dispersion of poly-β-benzyl-L-aspartate ▽—▽—▽ and poly-γ-benzyl-L-glutamate ⊙—⊙—⊙ in chloroform solutions over the wave length range 237 to 578 mμ. [Reproduced by permission of the American Chemical Society from R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).]

yield the desired data. As another approach to obtaining information about the relative helix sense in polypeptides and proteins I wish to describe an effect recently observed[14] by Lubert Stryer which is showing considerable promise.

It is well-known that proteins and other macromolecules bind dyes and we have been examining the binding of dyes to solutions of synthetic polypeptides. If basic dyes, such as Acriflavine or Acridine Orange, are added to solutions of polyglutamic acid, it is found that the polyglutamic acid binds the basic dyes over the pH range 4–7. At pH 7 polyglutamic acid exists mainly as the charged polyion and because of its charge this polypeptide attains random conformations at this pH. Measurements of the optical rotatory dispersion of basic dyes bound to polyglutamic acid in the random conformation show rotatory dispersions identical with that of the polypeptide

alone. Since the dyes contain no asymmetric center this finding is not surprising. However, if the optical rotatory dispersion is measured on solutions of the dye:polypeptide complexes when the polypeptide is in a helical conformation, rotatory dispersion of the type shown in Fig. 8 is observed.

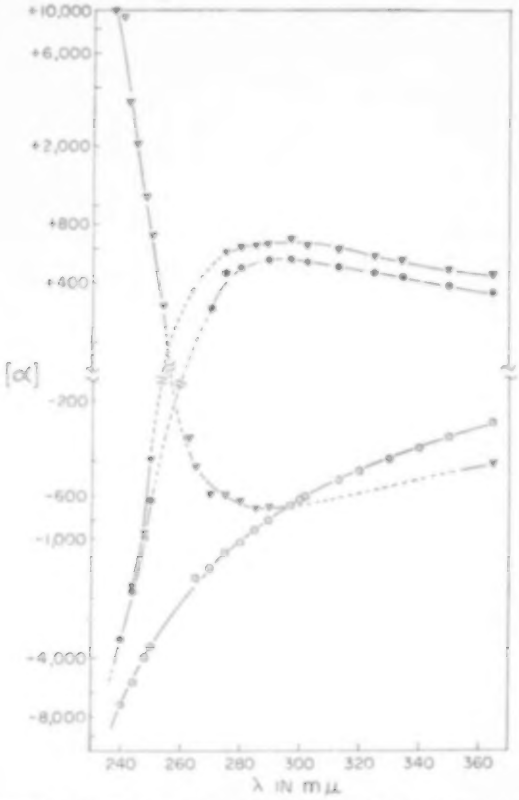


FIG. 7. Ultraviolet rotatory dispersions in chloroform solutions: ▽—▽—▽ poly-β-benzyl-L-aspartate; ○—○—○ 9:1 co-polymer of β-benzyl-L-aspartate:γ-benzyl-L-glutamate; ▼—▼—▼ poly-β-benzyl-D-aspartate; ●—●—● 9:1 co-polymer of β-benzyl-D-aspartate:γ-benzyl-L-glutamate. [Reproduced by permission of the American Chemical Society from R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).]

When the polypeptide is in the helical conformation, markedly anomalous rotatory dispersions are observed in the wavelength regions corresponding to the dye absorption band. The observed anomalous rotatory dispersion of the dye:helical polyglutamic acid complex is the Cotton effect. The observation of the Cotton effect under these conditions indicates that the chromophoric group of the dye has acquired asymmetry, and the location of the inflection point in the rotatory dispersion curve indicates that the dye is the absorbing moiety responsible for the anomaly. Measurements of these inflection points show good agreement with the absorption maximum of the dye. Data on a number of dye:polypeptide complexes are shown in Table 1 (from Refs. 14 and 15).

[14] E. R. Blout and L. Stryer, *Proc. Natl. Acad. Sci.* **45**, 1591 (1959).
[15] L. S. Stryer and E. R. Blout, *J. Amer. Chem. Soc.* **83**, 1411 (1961).

We have recently investigated this phenomenon in some detail since the magnitude of the Cotton effect with dye:polypeptide complexes may serve as a useful measure of the helix content of polypeptides and proteins. To do this we have measured the Cotton effect of Acridine Orange:polyglutamic acid complexes as a function of pH over the range where polyglutamic acid changes from helix to random conformation. From preliminary data it appears that the dye:helical polypeptide Cotton effect is a good measure of the helix content in this system.

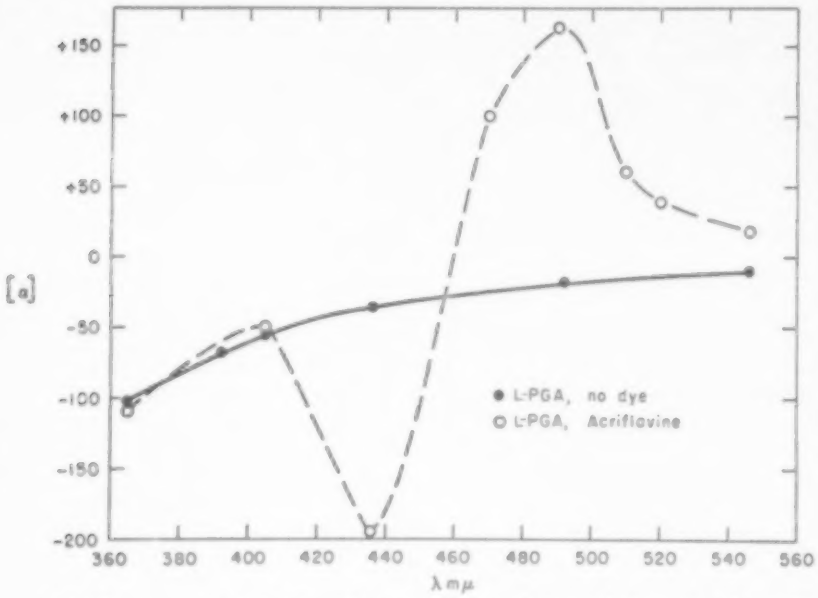
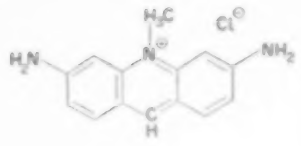
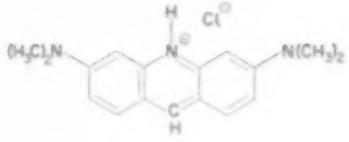
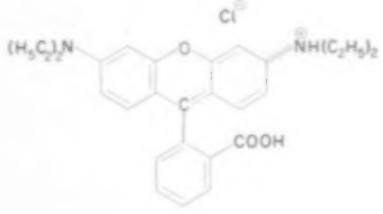
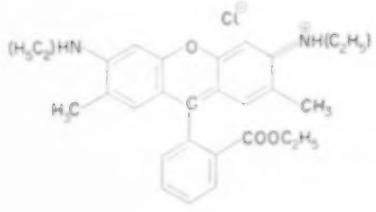
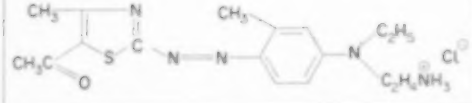


FIG. 8. – – – – – – The rotatory dispersion of an acriflavine:polyglutamic acid complex at pH 4·9. ————The rotatory dispersion of L-polyglutamic acid at the same pH. [Reproduced by permission of the National Academy of Sciences, Washington, D.C. from E. R. Blout and L. S. Stryer, *Proc. Natl. Acad. Sci.* **45**, 1591 (1959).]

It should be noted that a large Cotton effect can be obtained with various dye: residue ratios (see Table). In fact, definite and measurable Cotton effects have been obtained where the ratio of glutamic acid residues to Acridine Orange had a range between 20 to 1 and 4500 to 1.[15]

Since Acridine Orange shows a negative Cotton effect with L-polyglutamic acid and since L-polyglutamic acid molecules presumably have only one sense of twist, it was of interest to inquire what the effect of a polypeptide substrate of the opposite sense of twist would be on the rotatory dispersion of a dye:polypeptide complex. To examine this we chose the enantiomer of poly-L-glutamic acid, namely poly-D-glutamic acid. Whatever the absolute sense of twist of poly-L-glutamic acid, poly-D-glutamic should have the opposite sense of twist. The results of the optical rotatory dispersion measurements are shown in Fig. 9.[15] As can be seen the Acridine Orange:L-polyglutamic acid complex shows a *negative* Cotton effect whereas the Acridine Orange:D-polyglutamic acid shows a *positive* Cotton effect. Thus the Cotton effects observed with dye:

TABLE 1. SPECTRAL DATA ON THE BINDING OF SEVERAL BASIC DYES TO POLY-L-GLUTAMIC ACID

Dye	Dye structure	pH	Carboxyl: dye ratio	Inflection point of Cotton effect (in mμ)	Absorption maxima of dye (in mμ)
Acriflavine		4·9	185	458	457
Acridine Orange		4·5	915 2435	470 ~ 475	467 and shoulder at 497
Rhodamine B		4·7	1476	558 and 588	556 and shoulder at 585
Rhodamine 6G		4·6	2640 1320	491, 502, 533 492, 506, 537	496, 529 498, 530
Thiazole Magenta–T		4·5	190	480	478

polypeptide complexes may serve as a simple and effective method for the determination of the relative helix sense of polypeptides. Such measurements have now been extended to a variety of other dye types.[15]

Finally, encouraged by the above results with polypeptides, we have begun investigations of the rotatory dispersion of dyes bound to proteins. One protein which has

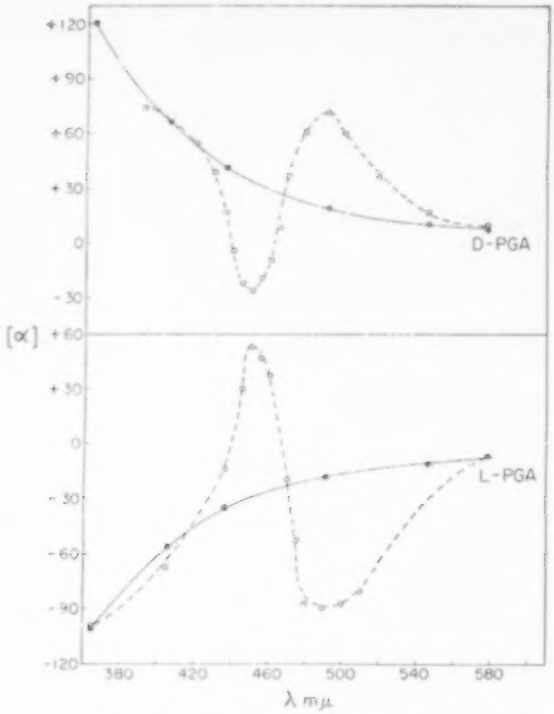


FIG. 9. – – – – – – The optical rotatory dispersion of acridine orange:D-polyglutamic acid complex (top figure), acridine orange:L-polyglutamic acid complex (bottom figure), at pH 4·7. ———— The rotatory dispersions of D- and L-polyglutamic acid respectively at the same pH. [Reproduced by permission of the American Chemical Society from L. S. Stryer and E. R. Blout, *J. Amer. Chem. Soc.* **83**, 1411 (1961).]

been studied is tobacco mosaic virus (TMV) and these experiments are being carried out in collaboration with Simmons[16]. When Acridine Orange is bound to TMV the rotatory data are anomalous and a Cotton effect is observed. If these data are compared with those obtained with Acridine Orange and polyglutamic acid in the presence of phosphate,[15] it is evident that the Cotton effect observed at 510 mμ has the same sign as that observed with the Acridine Orange:L-polyglutamic acid complex under the same conditions. Therefore the preliminary conclusion can be drawn that the sense of helix in TMV is the same as that in L-polyglutamic acid.

Acknowledgement—Much of the work reported here was supported in part by the Office of the Surgeon General, Department of the Army and Grant A2558 from the National Institutes of Health.

[16] N. Simmons, L. Stryer and E. R. Blout, to be published.

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OPTICAL ROTATION AND POLYPEPTIDE STRUCTURE*

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COMPARISON of experimental data on the optical rotation of polypeptides with an appropriate theoretical calculation can give a great deal of structural information about the polypeptide. However, there is at this time no theory which is known to be reliable. We would like to review what progress has been made along these lines.

The general quantum mechanical equation relating the average optical rotation to the electronic wavefunctions of a molecule was derived by Rosenfeld[1] in 1928.

$$[m'] = \left(\frac{3}{n^2+2}\right)\frac{[\alpha]M}{100}$$

$$[m'] = \frac{96\pi i N_0}{hc}\sum_n{}' \frac{\lambda_{0n}{}^2 \mu_{0n}\cdot \mathbf{m}_{0n}}{\lambda^2 - \lambda_{0n}{}^2} \qquad (1)$$

N_0 = Avogadro's number.
$i = \sqrt{-1}$
h = Planck's constant
c = speed of light
λ_{0n} = wave length corresponding to the transition from ground state 0 to excited state n.

The sum is over all excited states. The electric and magnetic dipole transition moments are defined as

$$\mu_{0n} = e\int \psi_0{}^* \sum_i \mathbf{r}_i\, \psi_n\, d\tau$$

$$\mathbf{m}_{0n} = \frac{e}{2mc}\int \psi_0{}^* \sum_i \mathbf{r}_i \times \mathbf{p}_i \psi_n\, d\tau$$

The wavefunctions refer to the entire molecule and the sums are over all the electrons in the molecule.

An approach to the calculation of the optical rotation can be made by considering the molecule to be an aggregate of discrete groups of electrons.[2a,b] To evaluate the transition moments the sum over electron is divided into sums over groups and the

* Supported in part by research grant A-2220 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, and by an unrestricted grant from Research Corporation.
† National Science Foundation Fellow.

[1] L. Rosenfeld, *Z. Physik.* **52**, 161 (1928).
[2a] J. G. Kirkwood, *J. Chem. Phys.* **5**, 479 (1937).
[2b] W. Moffitt, D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci.* **43**, 723 (1957).

molecular wavefunctions are written as products of group wavefunctions. The result can be written as

$$[m'] = \sum_{\text{groups}} \{g^0(V_{ij}, \boldsymbol{\mu}^i_{n_i 0}, \boldsymbol{\mu}^j_{n_j 0}, \mathbf{R}_{ij}) + g^1(V_{ij}, \boldsymbol{\mu}^i_{n_i 0}, \mathbf{m}^j_{n_j 0}, \mathbf{R}_{ij}) + [m_i']\} \qquad (2)$$

The potential V_{ij} is the interaction energy between the instantaneous charge distributions in groups i and j and $\mathbf{R}_{ij}$ is the distance between these groups. The electric and magnetic transition moments now refer to electrons, wavefunctions, and transitions in specific groups i and j. The optical rotation of group i is $[m'_i]$. It can be calculated from the effect of the time average fields of the other groups on group i. Moffitt[3] has shown that the value of g^1/g^0 is of order of magnitude $d/\mathbf{R}_{ij}$ where d is a particular distance within a group. That is, g^1 will be small relative to g^0 if the groups are small relative to the distance between them. The relative magnitude of $[m']$ to g^0 is difficult to assess. As the intensity of an absorption band is proportional to $\mu_{0n}{}^2$, we expect strong bands to contribute to the rotation mainly through g^0 while weak bands contribute through $[m_i']$.

Calculations for polypeptides have been made considering only the g^0 term. This allows the optical rotation as a function of wave length to be obtained from the geometry of the molecule and from measurable optical properties (absorption coefficients or polarizabilities) of the groups. The key assumptions are that there is no electron exchange between groups and that the interactions between groups is purely electrostatic. The validity of these assumptions will obviously depend on the choice of groups.

The α-helix

Moffitt[3,4] attempted to find the contribution of the amide groups to the optical rotation of an α-helix. He felt that the above assumptions were valid only for these non-bonded groups. The calculation involves determining the electronic interaction of each amide group with other amides in the polypeptide and with the incident light. For the interaction with the light the transient charge distribution of the excited amide can be characterized by the transition electric dipole moment. However, for the interaction with the nearest neighbor amides, Moffitt felt that the dipole approximation was inadequate. He used instead the transition monopoles at the N, C, and O atoms of the amide group. Both the mono- and di-pole moments were obtained from the spectrum of the amide group as determined by Peterson and Simpson[5]. Only transitions at 1850 and 1480 A were considered. Moffitt's final numerical result was incorrect because of an error in the choice of electronic wavefunction.

Fitts and Kirkwood[6] were willing to attempt a complete calculation of the optical rotatory dispersion of an α-helix with no side chains. They used the dipole approximation for the interaction between all groups, i.e., amide-amide, amide-CH, and CH—CH. However, they omitted interactions between adjacent atoms to approximately remove the contribution from asymmetric carbons. The necessary optical parameters were the transition dipole moments of the amide and the polarizabilities of the CH and the amide.

[3] W. Moffitt, *J. Chem. Phys.* **25**, 467 (1956).
[4] W. Moffitt, *Proc. Natl. Acad. Sci.* **42**, 736 (1956).
[5] D. L. Peterson and W. T. Simpson, *J. Amer. Chem. Soc.* **79**, 2375 (1957).
[6] D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci.* **43**, 723 (1957).

Tinoco and Woody[7] extended the calculations of Fitts and Kirkwood to include polyglycine and polyalanine. They also calculated the optical activity for light incident parallel to the helix axis of the polypeptide besides the average rotation. In the Kirkwood approximation the general equation for the effective molecular rotation can be written as

$$[m'] = \sum_i \sum_{j>i} \frac{\alpha_i \alpha_j A_{ij}}{\lambda^2} \tag{3}$$

where α_i, α_j are the polarizabilities of groups of type i and j; A_{ij} is a constant depending mainly on the geometry of the molecule and the direction dependence of the polarizabilities. For a peptide with hydrocarbon side chains we write

$$\alpha_{\text{amide}} = \frac{A_1\lambda^2}{\lambda^2 - \lambda_1^2} + \frac{A_2\lambda^2}{\lambda^2 - \lambda_2^2} + A_3$$

$$\alpha_{\text{hydrocarbon}} = A_4$$

then

$$[m'] = \frac{a_{11}\lambda^2}{(\lambda^2 - \lambda_1^2)^2} + \frac{a_{12}\lambda^2}{(\lambda^2 - \lambda_1^2)(\lambda^2 - \lambda_2^2)} + \frac{a_{22}\lambda^2}{(\lambda^2 - \lambda_2^2)^2} + \frac{a_{10}}{\lambda^2 - \lambda_1^2} + \frac{a_{20}}{\lambda^2 - \lambda_2^2} + \frac{a_{00}}{\lambda^2} \tag{4}$$

The first three terms represent the mutual interactions of the 1850 A (λ_1) and 1480 A (λ_2) amide transitions. The next two terms include the interaction of these two special transitions with the rest of the polypeptide. The last term is the contribution from the constant polarizabilities. All six terms are of the same order of magnitude and can be positive or negative. There will obviously be considerable uncertainty in the magnitude of the final calculated rotation. The results are given as the solid lines in Figs. 1, 2, and 5.

The pure L *polymer.* Fig. 1 gives the calculated average rotation for (1) right-handed poly-L-alanine and (5) left-handed poly-L-alanine. The broken lines show experimental results for (2) polybenzyl-L-glutamate in ethylene dichloride,[8] (3) poly-L-glutamic acid in dioxane-H_2O,[8] (4) poly-L-leucine in benzene-*m*-cresol,[9] and (6) poly-L-alanine as solid film.[10] The general agreement between experiment and calculation for the left-handed α-helix is striking.

The D,L *copolymer.* Fig. 2 gives the calculated average rotation for (2) right-handed polyglycine omitting adjacent atoms,[11] (4) right-handed poly-DL-alanine and (5) left-handed polyglycine. The broken lines show experimental results for helices with the sense of the L isomers of (1) polybenzyl-DL-glutamate in dioxane[12] and (3) poly-DL-leucine in benzene-*m*-cresol.[9] The comparison in this figure favors right-handed helices, as previously concluded by Fitts and Kirkwood, but is in contradiction to our conclusion from Fig. 1. As the experimental results were found by various extrapolation procedures, it is well to consider these carefully.

[7] I. Tinoco, Jr. and R. W. Woody, *J. Chem. Phys.* **32,** 461 (1960).
[8] W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci.* **42,** 596 (1956).
[9] A. R. Downie, A. Elliott, W. E. Hanby and B. R. Malcolm, *Proc. Roy. Soc.* A **242,** 325 (1957).
[10] A. Elliott, W. E. Hanby and B. R. Malcolm, *Disc. Faraday Soc.* **25,** 167 (1958).
[11] This is the model used by Fitts and Kirkwood. Their calculated curve agrees within experimental error with curve (1).
[12] P. Doty and R. D. Lundberg, *Proc. Natl. Acad. Sci.* **43,** 213 (1957).

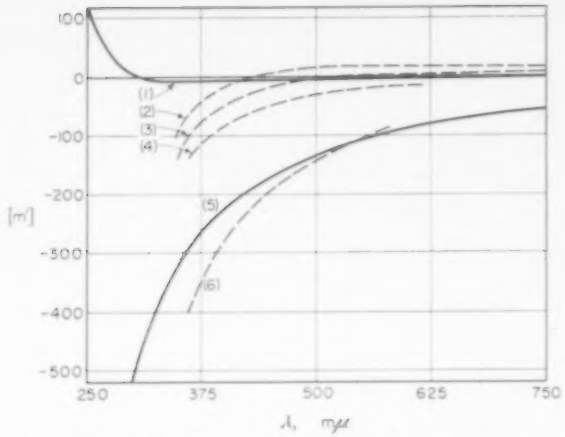

FIG. 1. The average optical rotation vs. wavelength. The solid curves are calculated: (1) right-handed poly-L-alanine, (5) left-handed poly-L-alanine. The broken curves are experimental: (2) polybenzyl-L-glutamate in ethylene dichloride,[8] (3) poly-L-glutamic acid in dioxane-water,[8] (4) poly-L-leucine in benzene-*m*-cresol,[9] (6) poly-L-alanine as solid film.[10]

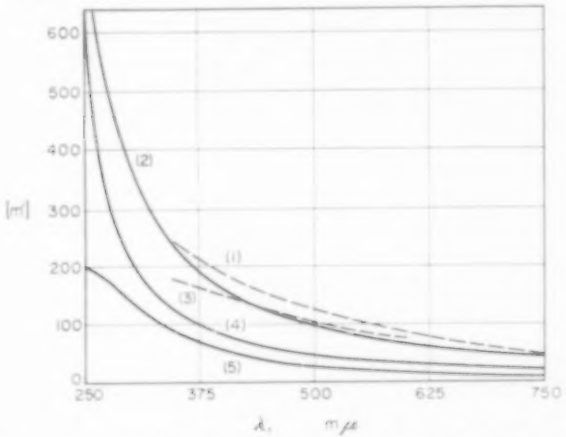

FIG. 2. The average optical rotation vs. wave length. The solid curves are calculated: (2) right-handed polyglycine (omitting adjacent atoms), (4) right-handed poly-DL-alanine, (5) left-handed polyglycine. The broken curves are experimental: (1) polybenzyl-DL-glutamate in dioxane,[12] (3) poly-DL-leucine in benzene-*m*-cresol.[9] These latter curves are for helices with the sense of the poly-L-isomer.

Measurements of the optical rotation of D,L polypeptides have been made in an attempt to determine the contribution of the α-helix to the rotation.[13] A typical experiment[9] is shown in Fig. 3 where the observed rotation for a polypeptide in a helix solvent is plotted vs. f_D, the mole fraction of D monomers in the polymerizing system. By extrapolation of the initial linear portion of curve to $f_D = 0{\cdot}5$, one obtains the "rotation of the α-helix with racemic residues." Let us examine what this means.

All theories of optical rotation agree that the rotation is due to the interaction between groups. We can distinguish between groups in the covalently bonded backbone

[13] An alternative experiment might be to form co-polymers with glycine and extrapolate to 100% polyglycine

BB and those in the side chains L or D and therefore write quite generally for the rotation:

$$[m'] = (BB) + f_L(L - BB) + f_D(D - BB) + f_D^2(D - D) + f_L^2(L - L) + 2f_Df_L(D - L) \quad (5)$$

The contribution from the backbone is (BB); (L − BB) is the contribution from the interaction between L side chains and backbone, etc. We can think of equation (5) as

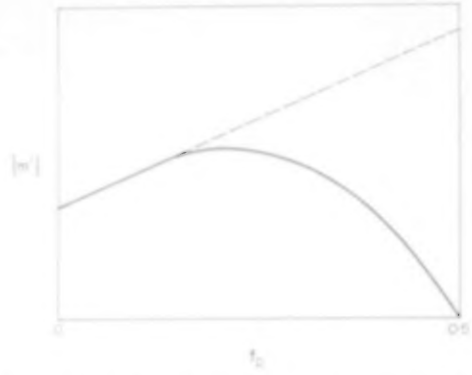

FIG. 3. The optical rotation for a polypeptide in a helix solvent is plotted vs. f_D, the mole fraction of D monomers in the polymerizing system. By extrapolation (broken line) of the initial linear portion of the curve to $f_D = 0{\cdot}5$, one obtains the "rotation of the α-helix with racemic residues."

representing the solid line in Fig. 3. The equation for the broken line used in extrapolation can then be written

$$[m']^{DL} = [m']^{L} + \left(\frac{\partial[m']}{\partial f_D}\right)_{f_D \to 0} f_D \quad (6)$$

The form of this equation (and therefore the extrapolated value $[m']^{DL}_{f_D=0.5}$) will depend on the assumptions made about the effect of D substitution on the backbone. These could be:

(1) Helix backbone is unchanged by slight substitution of D residues. The extrapolated value for the helix with racemic side chains becomes

$$[m']^{DL}_{f_D=0.5} = (BB_L) + 0{\cdot}5(D - BB_L) + 0{\cdot}5(L - BB_L) + (D - L) \quad (7)$$

The subscript L on BB means that the backbone rotation is that of the pure L isomer.

(2) Helix sense is directly proportional to the mole fraction of each isomer.

$$[m']^{DL}_{f_D=0.5} = (BB_L) - (D - L) \quad (8)$$

(3) Helix backbone is distorted by slight substitution of D residues. This means we must add the following terms to equation (7)

$$0{\cdot}5\left[\frac{\partial(BB_L)}{\partial f_D} + \frac{\partial(L - BB_L)}{\partial f_D} + \frac{\partial(L - L)}{\partial f_D}\right] f_D \to 0$$

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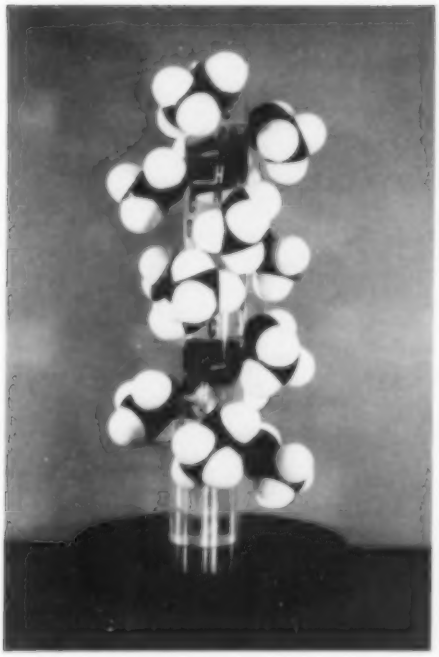

FIG. 4. A left-handed α-helix of poly-L-alanine with one D residue. Note the steric hindrance between the D and L methyls.

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These terms represent the linear change in rotation due to the distortion of the helix by the addition of D residues.

It is clear that the significance of the extrapolated value depends strongly on what actually happens to the backbone helix when D residues are added. The experimental workers have assumed that the helix backbone is unaffected by slight D substitution, therefore they should favor equation (7). As $(D - BB_L)$, the contribution of the interaction of D side chains with the backbone helix corresponding to the L isomer, is *not* equal to minus $(L - BB_L)$, this extrapolation does *not* give the rotation of a helix without side chains. For polyalanine we have calculated $(D - BB_L)$ to have the same sign and order of magnitude as $(L - BB_L)$. The contribution $(D - BB_L)$ is of equal magnitude and *opposite* sign to $(L - BB_D)$. This is why assumption (2) (the inversion of the helix sense is directly proportional to D concentration) is necessary to give an extrapolated value which does not contain side chain-backbone interactions. Both cases (1) and (2) have contributions, which may be small, from the interaction of the side chains with each other, (D — L). The most reasonable assumption is probably (3), i.e. some helix distortion occurs every time a D residue is introduced. Models show that substitution of a D for an L residue in an α-helix creates enough steric hindrance to block completely rotation of the β carbons in both the D and interacting L side chains. In Fig. 4 is a picture of left-handed poly-L-alanine containing one D residue. The methyls in this model can barely rotate, but for all larger side chains rotation is not possible. The increase in enthalpy and decrease in entropy which this would cause could be offset by distortion and/or disruption of the helix.

The conclusion we draw from this discussion is that the optical rotation of D,L polymers is very difficult to interpret. Curve (4) in Fig. 2 is calculated for right-handed poly-DL-alanine on the basis of equation (7). Curve (5) for left-handed polyglycine is essentially equal to (BB) and so it is roughly a comparison to experiment based on equation (8). If assumption (3) is correct then neither of these comparisons is valid and Fig. 2 does not give us any information about the helix sense.

The oriented polymer. Fig. 5 gives the calculated rotation for light incident along the helix axis for (1) left-handed polyglycine, (2) left-handed polyglycine omitting adjacent atoms, (3) left-handed poly-DL-alanine, (5) left-handed poly-L-alanine, and (6) right-handed poly-L-alanine. The dotted lines show experimental results for polybenzyl-L-glutamate in (4a) dioxane[14] and (4b) ethylene dichloride.[15] We notice that all the calculated curves for left-handed helices are qualitatively the same and that they are in general agreement with the experiments.

The effect of side chains. All the calculations have been made for the simplest polypeptides, glycine and alanine, while most of the experiments have been done on more complicated polymers. The effect of the side chains on the optical rotation is difficult to calculate. One problem is in not knowing the configuration of the side chains. Two extreme examples are shown in Fig. 6. Fig. 6a shows left-handed polybenzyl-L-glutamate with the side chains arranged randomly, while Fig. 6b shows that an extremely non-random arrangement of the benzene rings is sterically possible. In spite of these difficulties it will probably be possible to estimate the effect of the various side chains on the optical rotation.

The effect of solvent. Calculations of the optical rotation have of course omitted

[14] K. Yamaoka, unpublished experiments.
[15] I. Tinoco, Jr., *J. Amer. Chem. Soc.* **81**, 1540 (1959).

solvent interactions. Theoretically we know they exist and there is experimental proof for the change is optical rotation in various helix solvents.[16] For our comparisons we can either use solvents which interact weakly and randomly with the polymer or else try to obtain a parameter which is independent of solvent. By making measurements near the amide absorption band one may find such a parameter.

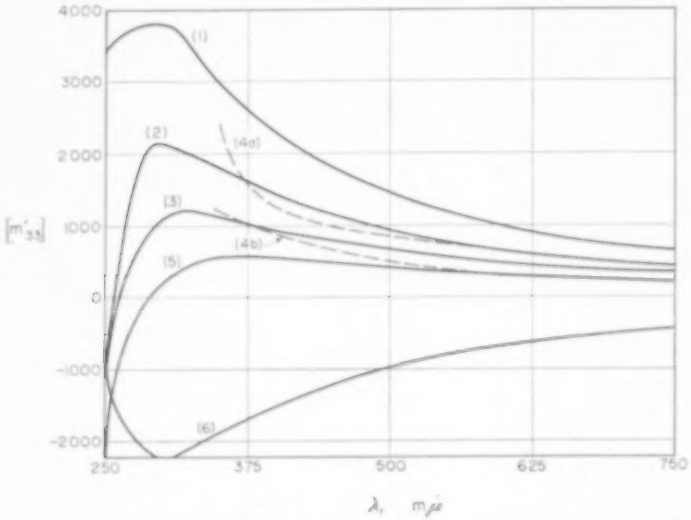

FIG. 5. The optical rotation for light incident along the helix axis vs. wave length. The solid curves are calculated: (1) left-handed polyglycine, (2) left-handed polyglycine (omitting adjacent atoms), (3) left-handed poly-DL-alanine, (5) left-handed poly-L-alanine, (6) right-handed poly-L-alanine. The broken curves are experimental: polybenzyl-L-glutamate in (4a) dioxane[14] and (4b) ethylene dichloride.[15]

Other measures of the sense of the helix. X-ray scattering would seem to be the definitive method of determining the sense of the helix. However, recent x-ray work has been contradictory. Arndt and Riley[17] made an extensive determination of x-ray powder diagrams of proteins and synthetic polypeptides. They claimed that in an amorphous solid protein, most of the detectable scattering would come from intramolecular distances. Therefore, they need not assume a particular intermolecular packing to interpret their results. They concluded that there were large amounts of left-handed α-helices (but not right-handed helices) in all their samples. Calculations have been made by Brown and Trotter[18] and Elliott and Malcolm[19] to compare with the same x-ray data on poly-L-alanine fibers.

Their conclusions have depended on the assumption on interchain packing. Brown and Trotter favored a left-handed helix with poor agreement between observed and calculated data, but Elliott and Malcolm with much better agreement definitely chose a right-handed helix. With the uncertainty in the packing still to be resolved we feel

[16] J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957).
[17] U. V. Arndt and D. P. Riley, *Phil. Trans. Roy. Soc.* A **247**, 409 (1955).
[18] L. Brown and I. F. Trotter, *Trans. Faraday Soc.* **52**, 537 (1956).
[19] A. Elliott and B. R. Malcolm, *Proc. Roy. Soc.* A **249**, 30 (1959).

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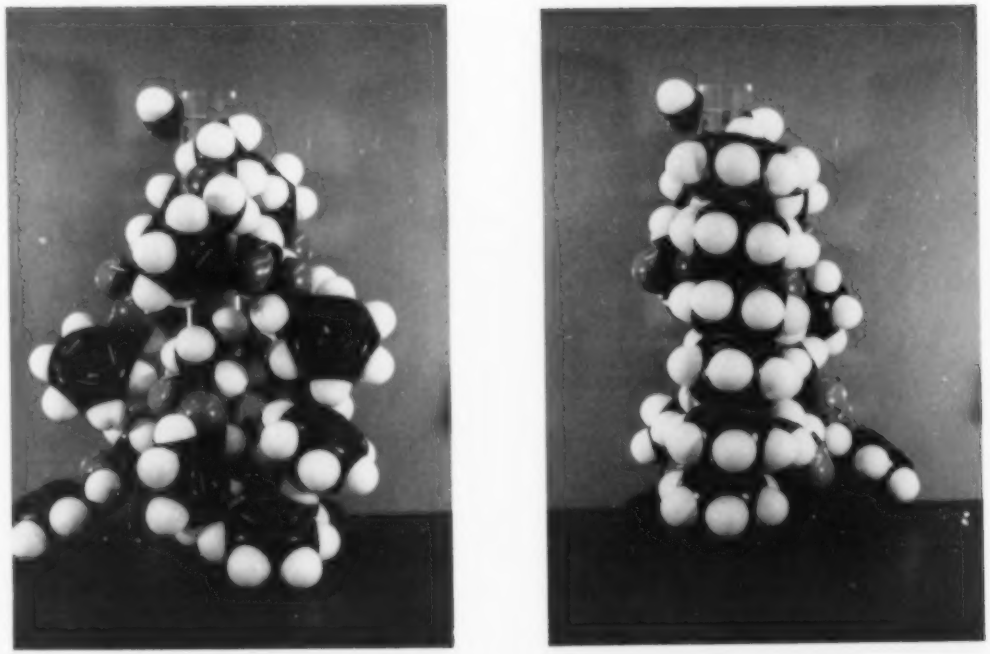

(a) (b)

FIG. 6. A left-handed α-helix of polybenzyl-L-glutamate in a (a) random and (b) ordered conformation.

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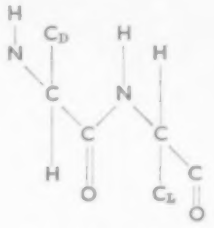
further evidence is desirable. It may be that the work of Kendrew *et al.*[20] on single crystals will provide this evidence.

Another approach to the problem of the sense of the helix is by consideration of the intramolecular forces which determine whether the L isomer forms a right or left-handed helix. Huggins[21] proposed that short range repulsive forces were dominant. This can be illustrated by the following sequence for a left-handed helix.



For a right-handed sequence the C_L and C_D would be interchanged. The distance from the β carbon in the L side chain C_L to the adjacent carbonyl oxygen is 2·7 A. Huggins thought this distance too short and therefore favored the C_D position; that is he thought that the L isomer should form right-handed helices. Donohue[22] has pointed out that identical distances between non-bonded O and C have been found in various amino acid crystals and thus rejects the argument.

We have been looking at the attractive forces between non-bonded atoms in the polypeptide. The important forces determining the relative stability of the right- and left-handed helix are permanent dipole forces and London forces between side chains and backbone. The dipole forces vary as r^{-3}, but can be attractive or repulsive, while the London force is always attractive and varies as r^{-6}. Our calculation for polyalanine which is still in progress, is analogous to one made by Pitzer and Catalano[23] to determine the relative heats of formation of isomeric hydrocarbons.

If Huggins' view is correct that steric repulsion of the β carbon determines the sense of the α-helix then all α-amino acid must form the same sense helix. However, if attractive forces are the main factor then each side chain can determine its preferred configuration. There is good evidence that not all polypeptides do have the same sense helix.[24a,b]

Other polypeptide structures

Very few calculations of optical rotation have been made for other polypeptide structures besides the α-helix. Murakami[25] has made calculations for oligopeptides of polyalanine. The random coil has not been explicitly considered. Zimm *et al.*[26] have used the continuous polarizability model of Fitts and Kirkwood[27] to show that the

[20] J. C. Kendrew in *Biophysical Science–A Study Program* (Edited by J. L. Oncley) p. 95. John Wiley, New York (1959). A personal communication from P. Doty has informed us that Kendrew has indeed found 70% right-handed helices in myoglobin.
[21] M. Huggins, *J. Amer. Chem. Soc.* **74**, 3963 (1952).
[22] J. Donohue, *Proc. Natl. Acad. Sci.* **39**, 470 (1953).
[23] K. S. Pitzer and E. Catalano, *J. Amer. Chem. Soc.* **78**, 4844 (1956).
[24a] E. R. Blout and R. H. Karlson, *J. Amer. Chem. Soc.* **80**, 4631 (1958).
[24b] E. R. Blout, *Tetrahedron* **13**, 123 (1961).
[25] H. Murakami, *Bull. Chem. Soc. Japan* **27**, 246 (1954); **28**, 583 (1955).
[26] B. H. Zimm, P. Doty and K. Iso, *Proc. Natl. Acad. Sci.* **45**, 1601 (1959).
[27] D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci.* **42**, 33 (1956).

optical rotation of a molecule containing short regions of helix and random coil is a linear function of the amount of each. Using the present, more realistic model of Fitts and Kirkwood[6] we find that the calculated optical rotation is a more slowly converging function of distance. Therefore more work is still needed to establish how many turns of a helix are essentially equivalent to an infinite helix.

Other types of helices and intermolecularly bonded structures have not been quantitatively considered although there is experimental evidence for the latter.

Conclusions

The goal of being able to determine the conformation of a polypeptide from its optical rotation has not yet been realized. There is a great deal of empirical knowledge about certain polypeptides, but the ability to generalize is not yet present. Current theories of optical rotation must be carefully tested; more calculations for polypeptides in various conformations and with various side chains are needed. For comparison we need measurements on simple polypeptides of known conformation. These measurements should be made in non-interacting solvents and particularly, at low wave lengths.

Note added in proof: An IBM 704 has been used to recalculate the results given as the solid lines in the figures. Numerical errors have thus been found in the original calculation (Ref. 7). The solid lines in Fig. 1 are incorrect; comparison of the new results with experiment do not lead to a choice of the sense of the helix. The remaining figures are not qualitatively changed by the recalculation.

Kendrew *et al.* [J. C. Kendrew, R. E. Dickerson, B. E. Strandberg, R. G. Hart and D. R. Davies, *Nature, Lond.* **185,** 422 (1960)] have conclusively found that polypeptides with the rotatory dispersion shown in Fig. 1 are right handed.

Acknowledgement—We wish to thank Mr. John Clark for making and photographing the molecular model shown in Figs. 4 and 6.

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OPTICAL ROTATORY DISPERSION OF POLYPEPTIDES AND PROTEINS

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"THE invention in 1866 of the Bunsen burner inhibited the more laborious study of rotatory dispersion by making it almost too easy to work with the nearly monochromatic light of the sodium flame, and in this way brought to an end the fertile era which Biot had inaugurated half a century before." Thus wrote Lowry in his classical study of optical rotatory power.[1] As a matter of fact, during the time of Biot and his famous pupil, Pasteur, nearly all studies of optical activity were associated with measurements of optical rotatory dispersion. It was indeed most unfortunate that much valuable information was lost in later years through the extensive use of the sodium lamp by almost all the workers in this field. Within the last five years, however, we have witnessed an increasing interest in the optical rotatory power, both theoretically and experimentally. With the availability of commercial spectropolarimeters, the optical rotatory dispersion studies have experienced a rebirth, and have already yielded many fruitful findings as a result of new developments. On the one hand, this technique has been extensively applied to the structural studies of organic compounds, as evidenced by the beautiful work of Djerassi *et al.*[2] Of equal importance are the applications of optical rotatory dispersion to the study of polypeptide and protein conformations. It is at the latter aspect that the present paper will be aimed. Already the vast interest in this field has resulted in the appearance of several excellent reviews.[3–6] A monograph on synthetic polypeptides has also been published which describes in detail the work of the English school.[7] Therefore (and taking into consideration the limits of time and space imposed upon me) it seems superfluous to discuss recently publishd work; nor is it necessary to describe the work of some of the distinguished participants present at this symposium. Rather, we will limit our brief review to some of our early work, which I, myself, had the good fortune to observe closely. Some of the older proposals and interpretations will also be re-examined in view of later developments.

The concept of optical rotations of α-helices

All proteins in aqueous solution exhibit levorotatory optical rotations. In general the specific rotations, $[\alpha]_D$ (Na D line), of most native proteins lie in the range of -20 to $-70°$, whereas upon denaturation this value is invariably lowered by 20–70° and

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[1] T. M. Lowry, *Optical Rotatory Power*. Longmans, Green, London (1935).
[2] C. Djerassi, *Optical Rotatory Dispersion*. McGraw-Hill, New York (1960).
[3] P. Doty, [a] *Coll. Czech. Chem. Comm.* **22**, 5 (1957); [b] *Rev. Mod. Phys.* **31**, 107 (1959); [c] in *Proceedings of the Fourth International Congress of Biochemistry* (Edited by O. Kratky) Symposium IX. Pergamon Press, New York (1959).
[4] E. R. Blout, *Optical Rotatory Dispersion* Chap. 17. McGraw-Hill, New York (1960).
[5] W. Kauzmann, *Ann. Rev. Phys. Chem.* **8**, 413 (1957).
[6] S. J. Leach, *Rev. Pure App. Chem. Austr.* **9**, 33 (1959).
[7] C. H. Bamford, A. Elliott and W. E. Hanby, *Synthetic Polypeptides*. Academic Press, New York (1956).

generally restricted to the range of −80 to −120° for what appear to be completely denaturated forms. These facts strongly suggest that some structural elements exist in the native proteins, and the denaturation process involves a conformational change that is common to almost all proteins. These structural elements must be optically active, and contribute to the specific rotations of the native proteins, and the final values upon complete denaturation probably represent the average residue rotations of proteins. Our first thoughts would naturally turn to Pauling and Corey's[8] α-helix and suggest it as one of the structural elements which is optically active. To account for the rotation behavior of proteins, the $[\alpha]_D$ contributions due to this backbone must be opposite in sign and superimposed on the intrinsic negative $[\alpha]_D$ values of the amino acid residues. (Collagen, which cannot form the α-helix, is an exception to this rule and will therefore not be further discussed in this paper.) This idea was first formally put forward by Cohen in 1955.[9] It was this idea rather than later theories which prompted us to investigate the optical rotatory dispersion of polypeptides and proteins. From the beginning it was realized that the mere measurements of specific rotations at any particular wave length would not provide a quantitative test for the foregoing hypothesis and a more convincing proof would have to come from the optical rotatory dispersions.

Drude equation The most frequent representation of optical rotatory dispersion is the well-known Drude equation[10,11]

$$[\alpha]_\lambda = \Sigma k_i/(\lambda^2 - \lambda_i^2) \tag{1}$$

where λ_i is the wave length of the i-th absorption band of the molecule and k_i a constant characteristic of the i-th band. In most cases, however, equation (1) can be approximated by a simple one-term Drude equation[12]

$$[\alpha]_\lambda = k/(\lambda^2 - \lambda_c^2) \tag{2}$$

provided that $|(\lambda_i^2 - \lambda_c^2)| \ll |(\lambda^2 - \lambda_i^2)|$. Our first thought was that the dispersion data of the α-helical polypeptides and native proteins can be fitted with a complex two-term Drude equation[12]

$$[\alpha]_\lambda = k_1/(\lambda^2 - \lambda_1^2) + k_2/(\lambda^2 - \lambda_2^2) \tag{3}$$

one of which represents the contributions due to the helical backbone and the other due to the intrinsic residue rotations. Furthermore, since k_1 and k_2 must be opposite in sign, an anomalous dispersion might be anticipated if $|k_1| > |k_2|$ and $\lambda_1 < \lambda_2$. It was even speculated that native proteins are made up of partial helices and partial non-helices with the result that the dispersion data obey a pseudo-simple Drude equation. Thus it was indeed gratifying to find that all these expectations were confirmed experimentally, although the use of equation (3) was discarded in view of the later theoretical developments.

[8] L. Pauling and R. B. Corey, *J. Amer. Chem. Soc.* **72**, 5349 (1950); L. Pauling, R. B. Corey and H. R. Branson, *Proc. Natl. Acad. Sci.* **37**, 205 (1951).

[9] C. Cohen, *Nature, Lond.* **175**, 129 (1955).

[10] P. Drude, *Lehrbuch der Optik* (2nd Ed.) Hirzel, Leipzig (1906).

[11] For an excellent review see also, J. A. Schellman, *Compt. Rend. Trav. Lab. Carlsberg, Sér. Chim.* **30**, 363 (1958).

[12] New terminology has recently been suggested to describe the dispersion curve of organic compounds.[8] Accordingly both the simple and complex dispersions would have been called "plain" curves. This latter term, however, seems to be inadequate to emphasize the anomalous behavior of α-helical polypeptides (see Fig. 1). Until a uniform terminology is devised and accepted by both organic and protein chemists, we have here retained the Drude definitions.

To avoid future confusion we have now used the symbol λ_c for the dispersion constant in the one-term Drude equation, as distinguished from the symbol λ_0 for the complex dispersion shown in equation (4).

Synthetic polypeptides

Early experimental work. To test the foregoing hypothesis we undertook a detailed study of the optical rotatory dispersion of poly-γ-benzyl-L-glutamate (PBLG), which was the only synthetic polypeptide available to us at that time. The results are reproduced in Fig. 1.[13] Earlier, Doty *et al.*[14] demonstrated convincingly that this polypeptide can exist in either a helical or randomly coiled conformation in solution. Invariably we found that the α-helical forms exhibited anomalous dispersion whereas that of

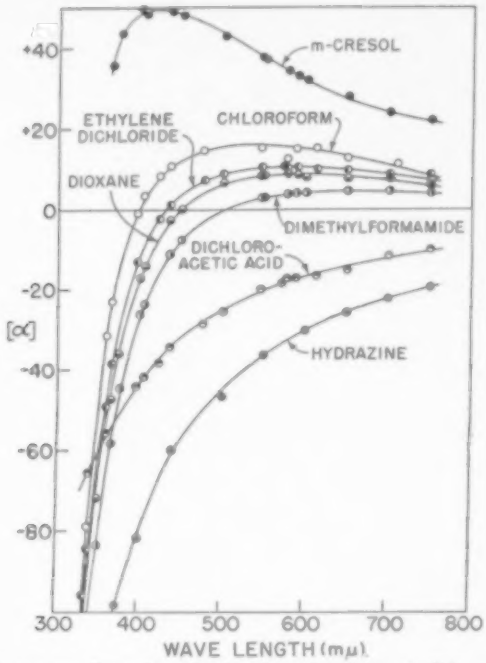

FIG. 1. Optical rotatory dispersion of poly-γ-benzyl-L-glutamate (Mw = 130,000) in several solvents at 20°C.

the coiled forms was always simple. These findings indicated clearly that the differences in rotation behavior were due in large part to the contributions of the structural elements in full agreement with our earlier expectations. Similar conclusions were also reached for poly-α-,L-glutamic acid (PLGA) the conformation of which can be converted into either form simply by adjusting its pH in aqueous solutions (Fig. 2).[15]

Theories. While this work was in progress theoretical developments of the α-helical optical rotation were also under way. Fitts and Kirkwood[16] applied Kirkwood's earlier polarizability theory[17] to the problem of calculating the contribution of an α-helix to the specific rotation $[\alpha]_D$ of a polypeptide. They subsequently found[18] that the

[13] J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957).
[14] P. Doty, A. M. Holtzer, J. H. Bradbury and E. R. Blout, *J. Amer. Chem. Soc.* **76**, 4493 (1954); P. Doty, J. H. Bradbury and A. M. Holtzer, *Ibid.* **78**, 947 (1956).
[15] P. Doty, A. Wada, J. T. Yang and E. R. Blout, *J. Polymer Sci.* **23**, 851 (1957).
[16] D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci.* **42**, 33 (1946).
[17] J. G. Kirkwood, *J. Chem. Phys.* **5**, 479 (1937).
[18] D. D. Fitts and J. G. Kirkwood, *J. Amer. Chem. Soc.* **78**, 2650 (1956).

calculated $[\alpha]_D$ due to the helical core agreed very well with the experimental difference in $[\alpha]_D$ between the helical and coiled forms from our first published data on PBLG.[19] This agreement, however, proved fortuitous since later results showed a moderate solvent effect on the helical form but a pronounced one on the coiled form.

Independently Moffit[20] also developed a theory of optical rotation using a quantum mechanical treatment. He was able to express the specific rotation of the helices in two terms

$$[m']_\lambda = \left(\frac{M_0}{100} \cdot \frac{3}{n^2+2}\right)[\alpha]_\lambda = \frac{a_0\lambda_0^2}{\lambda^2 - \lambda_0^2} + \frac{b_0\lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} \qquad (4)$$

where M_0 is the molecular weight per residue, n is the refractive index of the solvent, and a_0, b_0 and λ_0 are three constants. By multiplying both sides of equation (4) with

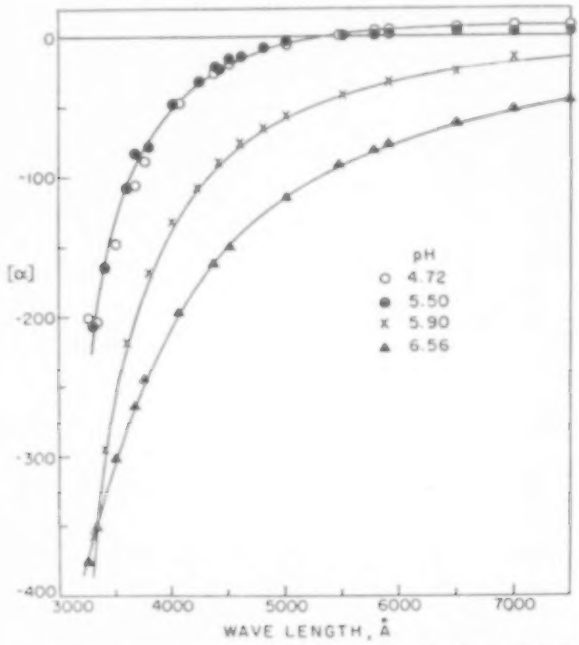

FIG. 2. Optical rotatory dispersion of poly-D,L-glutamic acid (Mw = 34,000) in 0·2 M NaCl-dioxane (2:1 by volume) at several pH values at 20°C.

$(\lambda^2 - \lambda_0^2)$ and by plotting $(\lambda^2 - \lambda_0^2)\,[m']_\lambda$ against $1/(\lambda^2 - \lambda_0^2)$ with λ_0 as a parameter, one obtains a straight line if a correct λ_0 value is chosen. Moffit's equation immediately found a great success in its application to the experimental data on PBLG and PLGA under various conditions.[21] This prompted him to make more theoretical calculations which gave $b_0 = -580$ and $\lambda_0 = 0{\cdot}200\ \mu$ for a right-handed α-helix,[22] as compared with the experimental values of $b_0 = -630$ and $\lambda_0 = 0{\cdot}212\ \mu$. Fitts and Kirkwood[18], however, have stated that the same type of dispersion as Moffitt's can be obtained from their theory, but their proposal leads to the second term in equation (4) having a

[19] P. Doty and J. T. Yang, *J. Amer. Chem. Soc.* **78**, 498 (1956).
[20] W. Moffitt, *J. Chem. Phys.* **25**, 467 (1956).
[21] W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci.* **42**, 596 (1956).
[22] W. Moffitt, *Proc. Natl. Acad. Sci.* **42**, 736 (1956).

positive sign, instead of a negative b_0 value, although both theories have predicted a right-handed α-helix for the polypeptide. It seems unnecessary to repeat in detail the story of these early developments although at that time it was somewhat embarrassing to us, since both workers had used our experimental data to support their theories, and Moffitt was gracious enough to insist on my being a joint author in one of his papers. Fortunately this friendly controversy came to a happy ending with the publication of a joint paper by the three theoreticians.[23] On the other hand, equation (4) became less attractive than when first proposed, since an error was found in Moffitt's earlier theory which nullified the numerical agreement between his equation and the experimental data. Neither can we now predict the screw sense of the helices as mentioned previously. It becomes clear that equation (4) is oversimplified and a rigorous treatment of this problem requires the introduction of additional trms. Fitts and Kirkwood[24] have evaluated these terms and found an impressive agreement with Doty and Lundberg's data on the rotatory dispersion of helical backbone.[25] However, it is difficult to judge whether the agreement is again fortuitous, since numerically large terms of opposite sign are involved in the calculations. Very recently Tinoco[26] has made a more refined treatment of the problem which has been discussed by him in this symposium (p. 134).

For practical purposes equation (4) should be regarded as an emprical equation, but a very useful one for characterizing the rotatory dispersion of helical polypeptides, especially since the refined theoretical treatments are not easy to apply. It may also be said that an alternative phenomenological equation in a form identical to equation (4) can be obtained by expanding equation (1) in inverse powers of $(\lambda^2 - \lambda_0^2)$.[21] Kauzmann[5] and Schellman and Schellman[27] have also pointed out that such an equation can be derived in other ways. In fact one may argue that the fit of equation (4) does not necessarily mean the presence of an α-helix. For instance, even simple amino acids can show anomalous dispersion if powerful chromophores are present. However, the origin of the rotatory dispersion by helices appeared to have been satisfactorily established by the various theoretical treatments.

To look back, we have indeed witnessed a most exciting development in the field of optical rotatory dispersion during the past five years. As we are gathered here today to review the progress, we are saddened by the untimely loss of two great scientists. It was a doubly black event when both Professors Kirkwood and Moffitt passed away within one year. And it is most appropriate that this symposium is dedicated to both of them. Needless to say, their contributions in this field have already opened up a new attack on this complicated problem and their work will be remembered for years to come.

Optical rotations at shorter wave lengths. In the application of equation (4) to the polypeptides, questions may be raised as to the nature of the absorption bands which are responsible for the anomalous dispersion. The origin of the complex dispersion may even be suspected to be due to the contribution of the broad band near 2600 Å of the chromophoric side chains such as the benzyl group in PBLG and that of the complex excitation band of the backbone helix at about 2000 Å. This ambiguity can be discounted by the fact that the α-helical PLGA which contains no aromatic groups also

[23] W. Moffitt, D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci.* **43**, 723 (1957).
[24] D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci.* **43**, 1046 (1957).
[25] P. Doty and R. D. Lundberg, *Proc. Natl. Acad. Sci.* **43**, 213 (1957).
[26] I. Tinoco, Jr. and R. W. Woody, *J. Chem. Phys.* **32**, 461 (1960).
[27] C. Schellman and J. A. Schellman, *Compt. Rend. Trav. Lab. Carlsberg, Sér. Chim.* **30**, 463 (1958).

exhibited the same complex dispersion.[15] Additional evidence comes from the dispersion measurements in the vicinity of the bands in question. For PBLG in ethylene dichloride only a slight deviation from the values based on equation (4) was observed in the range 2480 to 3130 Å.[13,28] In fact all the data could be linearized[28] by assigning to λ_0 a value of 0·208 μ instead of 0·212 μ. Also Dr. Blout has reported in this symposium (p. 123) no deviation at all for PBLG in chloroform. At any rate, no one has observed a Cotton effect near the chromophoric band of, say, the benzyl group.

Next, let us consider the choice between equation (4) and a two-term Drude equation. As a matter of fact, PBLG in ethylene dichloride (EDC) could also be fitted with equation (3), using $k_1 = 13{\cdot}9$, $k_2 = -8{\cdot}0$, $\lambda_1 = 0$ and $\lambda_2 = 0{\cdot}282$ μ. Previously the use of Moffitt's equation was preferred because of its theoretical basis and also because of the simplicity of its graphical method. It should also be added that the manual solution of the four simultaneous equations for the two-term Drude equation (1) is usually not unique. If three of the constants are arbitrarily specified it is not difficult to calculate the fourth constant and fit them with the existing experimental data over the wave length range employed. On the other hand, since equation (4) is now considered empirical, there is no reason why one cannot include the higher terms of the power series of $1/(\lambda^2 - \lambda_0^2)$, although the introduction of more constants makes it rather impractical to apply. This difficulty may be disposed of by a computing program, but we must still decide whether much can be gained through this modification.

The λ_0 value of 0·212 μ also deserves further consideration. Our early work covered a wave length range of 3500 to 7500 Å and the readings near the lower end of the scale were frequently obtained by using a wide slit on the monochromator and thereby introducing some errors due to impurity of the wave bands chosen. Nevertheless, it has been shown that the graphic solution of equation (4) was extremely sensitive to the chosen parameter λ_0.[21] Now with a better light source it is possible to make measurements below 3500 Å. As has already been mentioned, Doty and Savitz[28] have found a λ_0 value about two per cent lower than 0·212 μ. With this new value slight deviations from the straight line of the dispersion plot [equation (4)] should be expected for our early data near and about 3500 Å. It is important to note that such small deviations frequently escape detection in a graphic solution. A more reliable test will be to calculate the specific rotations according to equation (4) using the chosen λ_0 value and compare them with the experimental data. Only in this way can one determine the extent of any deviations. Suffice it to say that the λ_0 value should be more carefully examined with the accumulation of new data. Neither should one be led to think that the λ_0 value for one polypeptide will automatically be applicable to all other polypeptides, unless careful analyses of all existing experimental data so indicate. The question of whether equation (4) should be modified by including high terms of the power series or merely by using a new λ_0 value to cover the entire range of wave lengths of 7500 to below 2500 Å must first be clarified by re-examining the experimental data of various polypeptides. Until this is done we will continue to use equation (4) with λ_0 set at 0·212 μ in our discussion because of its simplicity and also its successful applications to our existing data on polypeptides. In this respect it is also interesting to note that the PBLG in its randomly coiled form obeys a one-term Drude equation with a λ_c value of 0·212 μ. The equivalence of λ_c and λ_0 values in equation (4) makes it extremely simple in the estimation of helical content as will be shown in a later section.

[28] P. Doty and D. Savitz, unpublished work.[29]

Side group interactions. In the theoretical treatment no allowance has been made for the interactions between side chains and helical backbone. It soon became apparent, however, that while some helical polypeptides such as poly-L-leucine,[29] poly-L-alanine,[30] and poly-α,L-lysine[31] did have the same b_0 and λ_0 values as the poly-α,L-glutamic acid, others such as poly-L-tyrosine,[32,33] poly-β-benzyl-L-aspartic acid[34] and poly-γ-benzyl-L-histidine[35] seemed to behave "abnormally". With the exception of poly-γ-benzyl-L-histidine all the dispersion data could also be fitted with equation (4) with λ_0 set at 2120 Å, but the b_0 values varied not only in magnitude but also in sign from −600 to +600. We are thus confronted with this question: Does this change in sign and magnitude of the b_0 values reflect the difference in helical screw senses or in conformations, or simply the contributions due to side group interactions? The first two possibilities seemed to be ruled out at least in the case of poly-L-tyrosine. On the basis of rotation studies coupled with other physical measurements, Coombes *et al.*[33] concluded that this polypeptide existed as helices near its solubility limit. From copolymerization studies these authors also eliminated the possibility of mixed helices. This left only the third possibility, i.e. the side group interactions. Very likely the proximity of such residues as tyrosine to the peptide bond causes close coupling between the two chomophores. The imposition of a second helical frame of the chromophoric groups on the helical backbone results in new pair-wise interactions which significantly modify the dispersion behavior.

Very recently Schellman and Schellman[27] have discussed in detail the effect of β-substituent on the optical rotations of amino acids (see also this symposium, p. 176), and Blout[4] has further classified the polypeptides into three classes. In Group I an optically inactive saturated hydrocarbon group is attached to the β-carbon of the amino acid residue, for example, glutamic acid. In Group II, the β-carbon substituent is any group other than $—CH_2—$, such as tyrosine, aspartic acid and histidine. Proline, hydroxyproline and their derivatives belong to Group III, which is actually a subdivision of Group II. These two amino acids are more appropriately called imino acids. The proline-containing polypeptides can not form an α-helical conformation due to the absence of the $—CO \cdots HN—$ type hydrogen bonds and therefore are considered in a separate group. This group is an interesting subject in itself, but we will not further elaborate on it in this paper. It is significant to note that all Group II polypeptides have strong side chain interactions and their dispersion behavior is strikingly different from that of Group I. For a co-polymer of Groups I and II, Blout *et al.*[36] have found that the Group I component is the dominating factor. This conclusion is most important in the interpretation of the optical rotations of proteins, as will become clear in a later section.

The screw sense of the helix. An α-helix can have two senses of twist. The right- and left-handed polypeptide helices are non-superimposable and, with the exception of polyglycine, they are not even the mirror images of each other. Thus, two problems

[29] A. R. Downie, A. Elliott, W. E. Hanby and B. R. Malcolm, *Proc. Roy. Soc.* A **242**, 325 (1957).
[30] A. Elliott, W. E. Hanby and B. R. Malcolm, *Nature, Lond.* **180**, 1340 (1957).
[31] J. Applequist, PhD. Dissertation, Harvard University (1958); J. Applequist and P. Doty, *Abstr. Amer. Chem. Soc. Meeting*, San Francisco, April (1958).
[32] A. R. Downie, A. Elliott and W. E. Hanby, *Nature, Lond.* **183**, 110 (1959).
[33] J. D. Coombes, E. Katchalski and P. Doty, *Nature, Lond.* **185**, 534 (1960).
[34] E. R. Blout and R. H. Karlson, *J. Amer. Chem. Soc.* **80**, 1259 (1958).
[35] E. Katchalski, G. D. Fasman and E. R. Blout, *Abstr. Amer. Chem. Soc. Meeting*, San Francisco, April (1958).
[36] R. H. Karlson, K. S. Norland, J. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).

immediately follow: Does the polypeptide helix prefer only one screw sense over the other, and if so, which direction is predominant? The first question has been discussed in detail by Dr. Blout at this symposium (p. 123). Thus our brief review will again be limited to some of the early work. The first evidence which supported the one-handedness came from the specific rotation measurements of copolymers of the D- and L-isomers of γ-benzylglutamates[37] and leucines.[29] The results of poly-γ-benzyl-glutamates are illustrated in Fig. 3. As the ratio of D/(D + L) increased from zero to

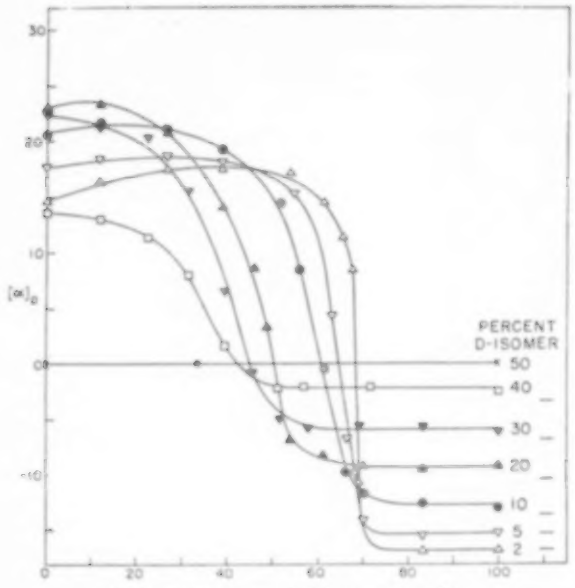

FIG. 3. Specific rotations (Na D line) of D-L copolymers of γ-benzylglutamate in chloroform-dichloroacetic acid at 20°C. The lines on the right indicate the rotation expected if rotations of D and L-isomers are additive. The data for the pure L-polypeptide have been omitted, because they fall so close to the polymer containing 2% D-isomers.

0·5, the $[\alpha]_D$ values of the coiled form (in dichloroacetic acid) changed proportionately toward zero as expected. The behavior of the helical form (in dioxane) was strikingly different, where the specific rotation actually rose to more positive values first and then decreased toward zero. This clearly implied that the helical polypeptide retained one screw sense with the introduction of the first portions of D-isomers. As a result the residue rotations of the L- (negative $[\alpha]_D$) and D- (positive $[\alpha]_D$) isomers, but not the backbone rotations, were largely cancelled out. When, however, the L/(D + L) ratio was less than 0·7, the helices were no longer of one screw sense. In fact their conformation gradually became unstable, as evidenced by the shift of the helix-coil transition zone toward the left in the figure. With these encouraging results, Doty and Lundberg[25] made a series of co-polymers by initiating a mixture of D- and L-anhydrides with poly-γ-benzyl-L-glutamate in a helix-promoting solvent. By linear extrapolation of the date at L/(D + L) ratios above 0·7 they found that the rotatory dispersion of the true

[37] E. R. Blout, P. Doty and J. T. Yang, *J. Amer. Chem. Soc.* **79**, 749 (1957).

helix fitted very well with equation (4) having $b_0 = -500$ if λ_0 was again set at 0·212 μ, and the corresponding $[\alpha]_D$ value of the helical backbone turned out to be $+54°$, which, if converted to a mean protein residue weight of 110, became 108°. The same authors have further shown by kinetic studies[38] that the helix of, say, L-polypeptide would be interrupted and changed into the opposite sense of twist only when as many as four residues of D isomers were added in sequence. This probability appears, however, to be remote in the co-polymerization study when the L/(D + L) ratio was greater than 0·7. Thus it seems highly probable that the helices do exist only in one-handedness.

Having disposed of one difficulty, we now turn to the second question concerning the choice between a right- and left-handed helix. Unfortunately this problem is still unsettled, but an excellent review has been given by Dr. Tinoco at this symposium (p. 134). Our early work[13] assumed that the helix was probably right-handed. This choice was based purely on Moffitt's theory[20,22] which, as we know now, can not give us an unequivocal answer. Fitts and Kirkwood[24], however, basing their conclusion on the data of Doty and Lundberg[25], decided that the choice of a right-handed helix was probably correct. On the other hand, Tinoco's recent theory[26] again seems to prefer a left-handed helix. It is of interest to note that, quite unaware of the later rotation work, Huggins[39] has predicted from theoretical calculations that a right-handed helix is a more stable conformation than a left-handed one. From their X-ray study, Arndt and Riley[40] have indicated that a left-handed helix seemed to predominate in several globular proteins, although their calculations might be inconclusive. A more concrete answer may eventually come from the beautiful X-ray work on myoglobin by Kendrew *et al.*[41], using Perutz's "isomorphous replacement method".[42] The latest information[43] appears to show conclusively that the helical regions of this protein are indeed right-handed. If the work on hemoglobin by Perutz and his co-workers should reach the same conclusion, we shall then be able to say with more confidence that proteins indeed seem to prefer a right-handed sense of twist, although the possibility of mixed helices can not be ruled out completely as a result of the restrictions of tertiary structures. In this respect it is of interest to recall that the change in specific rotation accompanying denaturation is almost always in the same direction for all proteins, which would be difficult to explain if helices of one screw sense are predominant in certain proteins and those of opposing twist prevail in others. Exceptions to the above observation are indeed rare and might even be taken as evidence of an unusual conformation. A notable example is collagen which has $[\alpha]_D$ value of about $-300°$ in the native state as a result of a polyproline type helix rather than an α-helix having a screw sense opposite to those observed in α-helical polypeptides.

Estimation of the helical content in proteins

It has been found that the optical rotatory dispersions of almost all proteins, both

[38] R. D. Lundberg and P. Doty, *J. Amer. Chem. Soc.* **79**, 3961 (1957).
[39] M. Huggins, *J. Amer. Chem. Sci.* **74**, 3963 (1952).
[40] U. W. Arndt and D. P. Riley, *Phil. Trans. Roy. Soc.* A **247**, 409 (1955).
[41] J. C. Kendrew, G. Bodo, H. M. Dintzis, R. C. Parrish and H. Wyckoff, *Nature, Lond.* **181**, 662 (1958); J. C. Kendrew, *Ibid.* **182**, 764 (1958); M. M. Bluhm, G. Bodo, H. M. Dintzis and J. C. Kendrew, *Proc. Roy. Soc.* A **246**, 369 (1958).
[42] D. W. Green, V. M. Ingram and M. F. Perutz, *Proc. Roy. Soc.* A **225**, 287 (1954).
[43] This information was kindly provided by Professor P. Doty. *Note added in proof:* See two recent papers on this subject: M. F. Perutz, M. G. Rossmann, A. F. Cullis, H. Muirhead, G. Will and A. C. T. North, *Nature, Lond.* **185**, 416 (1960); J. C. Kendrew, R. E. Dickerson, B. E. Strandberg, R. G. Hart, D. R. Davies, D. C. Phillips and V. C. Shore, *Ibid.* **185**, 422 (1960).

native and denatured, invariably obey a simple Drude equation. If the α-helix is a basic structural element in proteins, it is surprising that they show different dispersion behavior from that of the helical forms of synthetic polypeptides. To reconcile this apparent contradiction and in the absence of competing proposals we have set forth a working hypothesis[13] that (1) only a portion of the polypeptide chains of native proteins are folded into a helical conformation; (2) this structural element is essentially Pauling and Corey's α-helix as found in synthetic polypeptides and probably has the

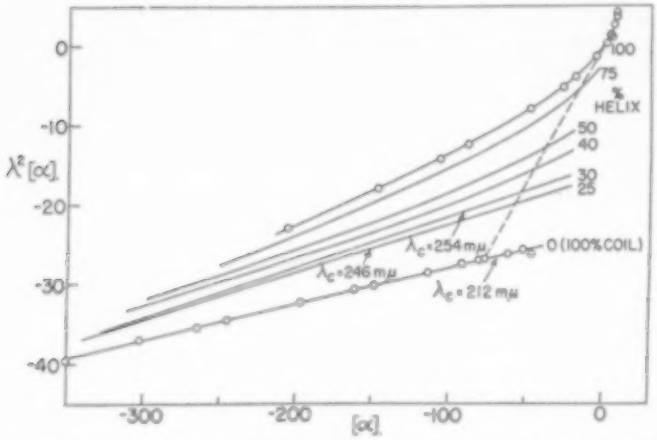

FIG. 4. Dispersion plots for poly-α,L-glutamic acid in the helical and randomly coiled forms together with combinations thereof in varying proportions. The broken line is drawn through the values at $\lambda = 5893$ Å.

same screw sense as that of the L-polypeptides; and (3) the nonhelical portions are irregular in the sense that their contributions to the optical rotation may be effectively the same as if they were random coils. (This irregularity, however, does not imply that the nonhelical polypeptide chains must be randomly coiled. In fact they may be just as rigid as the helices, but their patterns are non-periodical.) On the basis of the dispersion data of poly-α,L-glutamic acid in aqueous solutions, the dispersion curves of a series of hypothetical mixtures having both the helical (at pH 4·7) and coiled (at pH 6·6) forms were constructed.[13] The results are shown in Fig. 4, where $\lambda^2[\alpha]_\lambda$ is plotted against $[\alpha]_\lambda$ rather than the customary $1/[\alpha]_\lambda - \lambda^2$ plot[13,44] This simple representation could satisfactorily explain all the existing phenomena of the optical rotations of proteins in aqueous solutions. First, with normal experimental accuracy, the dispersion of the partial helices is indistinguishable from the simple Drude type unless more than 40 to 50 per cent of the polypeptide chains are in the helical form. Secondly, in the range where the dispersion remains apparently simple the dispersion constant λ_c in equation (2) increases steadily from 0·212 μ to 0·268 μ as the helical content varies from zero to 40 per cent (ignoring the small deviation present in the latter case). This is in good agreement with the observation of Linderstrøm-Lang and Schellman[45] that the λ_c values of native proteins decreased upon denaturation. Thirdly, the $[\alpha]_D$ values become

[44] More recently Heller has also treated the Drude equation having more than one term. See W. Heller, *J. Phys. Chem.* **62,** 1569 (1958).

[45] K. Linderstrøm-Lang and J. A. Schellman, *Biochim. et Biophys. Acta* **15,** 156 (1954); J. A. Schellman and C. Schellman, *Arch. Biochem. Biophys.* **68,** 319 (1957).

less levorotatory as the fraction of helical form increases, which again is a well recognized fact for native and denatured proteins. On the strength of these findings we proceeded to make a semiquantitative estimation of the helical content of several common proteins by comparing either the λ_c or $[\alpha]_D$ of these proteins in aqueous solutions with the calibration curve as furnished in Fig. 4 (after the correction of $[\alpha]_D$ due to the difference in the average residue weight of the proteins [assuming 110] and that of the PLGA [129]). The $[\alpha]_D$ method is straightforward. By establishing the average specific rotations of about $-110°$ for the denatured protein and $+5°$ for the pure helix, one finds that

$$\text{per cent helical content} = 100\,(110 + [\alpha]_D)/115$$

It must be kept in mind that this scale of 115 units is subject to variation of as much as $\pm 20°$. If the $[\alpha]_D$ values for the completely denatured protein is known in a special case, the above formula would be accordingly modified. The λ_c method can empirically be deduced from the data in Fig. 4. An approximately linear plot of λ_c versus the per cent helical content yielded a slope of about 0·0014. Thus

$$\text{per cent helical content} = 100\,(\lambda_c - 0{\cdot}212)/0{\cdot}14$$

Alternately, Doty[46] has derived the following relationship

$$\text{per cent helical content} = 100\,(\lambda_c^2 - 0{\cdot}045)/0{\cdot}055$$

It is noted from Fig. 4 that the λ_c scale only runs as high as about 40 to 50 per cent since higher helical contents should give rise to anomalous dispersion in which case λ_c is no longer defined. Nevertheless, with actual experimental data, one could miss a small curvature in a $\lambda^2[\alpha]_\lambda - [\alpha]_\lambda$ plot and thereby obtain a λ_c value which corresponds to a helical content higher than 50 per cent. In such cases a rough estimate can still be made with due reservations. Dr. Jirgensons has indicated in this symposium (p. 166) that most denatured proteins show a λ_c value in the range of 0·215 to 0·220 μ. Thus, for proteins which behave according to our working hypothesis, the λ_c method seems to be able to provide a reasonable estimate of the helical contents.

Very recently Imahori *et al.*[3c,47] have further refined the treatment by incorporating equation (2) into equation (4), which becomes

$$[m']_\lambda \equiv \frac{M_0}{100} \cdot \frac{3}{n^2 + 2}\,[\alpha]_\lambda = (a_0^R + a_0^H)\,\frac{\lambda_0^2}{\lambda^2 - \lambda_0^2} + \frac{b_0\lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} \qquad (5)$$

Here $a_0^R\lambda_0^2/(\lambda^2 - \lambda_0^2)$ represents the contributions due to the intrinsic residue rotation and the remaining terms on the right side of equation (5) are simply Moffitt's dispersion equation for the α-helix. By using $\lambda_0 = 0{\cdot}212\ \mu$ and plotting $(\lambda^2 - \lambda_0^2)[m']_\lambda/\lambda_0^2$ against $\lambda_0^2/(\lambda^2 - \lambda_0^2)$ one can easily determine $(a_0^H + a_0^R)$ and b_0 graphically. Further, by determining a_0^R from separate measurements on the randomly coiled form one can calculate the a_0^H from the difference between $(a_0^R + a_0^H)$ and a_0^R. On the basis of the data of PLGA in aqueous solutions, Doty *et al.* have found that $a_0^H = 650$, and $b_0 = -630$ as already mentioned. There is reason to believe from the available data on polypeptides that these values may have an uncertainty of no more than ± 10 per cent. Thus we have two more methods for determining the helical contents of proteins.

[46] P. Doty, *Advanc. Protein Chem.* In press.
[47] K. Imahori, E. Klemperer and P. Doty, *Abstr. Amer. Chem. Soc. Meeting*, Miami, April (1957).

This is done by comparing the experimental a_0^H and b_0 values of proteins with the theoretical ones, that is,[48]

$$\text{per cent helical content} = 100a_0^H/650$$
$$\text{or} = -100\ b_0/630$$

noting that the a_0^R values of proteins can be estimated from their completely denatured forms. It is pertinent to note that a_0^R is strongly solvent-dependent. Thus these methods can hold only when the environment in the randomly coiled form closely matches that in the helix. The same rule is also applicable to the $[\alpha]_D$ and λ_c methods. This condition is approximately met when both native and denatured proteins are studied in aqueous solutions. Finally we may also mention a recent observation

TABLE 1. HELICAL CONTENTS OF VARIOUS PROTEINS IN WATER[3a,c]

Protein	$-100\ b_0/630$	$a_0^H/650$	$100([\alpha]_D + 110)/115$*	$100(\lambda_c^2 - 0{\cdot}045)/0{\cdot}055$†
Tropomyosin	88	87	91	
Insulin	38	57		
Bovine serum albumin	46	58	46	
Ovalbumin	31	50	53	(51)
Lysozyme	29	39	37	36
Pepsin	31	26		
Histone	20	30	40	
Ribonuclease	16	17	17	14

* Assuming that the $[\alpha]_D$ is $+5°$ for the pure helix and $-110°$ for the denatured protein.
† Assuming $\lambda_0 = 0{\cdot}212\ \mu$ and, thus, $\lambda_0^2 = 0{\cdot}045\ \mu^2$.[46]

by Blout and Stryer[49] that cationic dyes and helical polyglutamic acids interacted and showed the Cotton effect in the dispersion measurements. Dr. Blout has also suggested in this symposium (p. 123) that the dependence of the observed circular dichroism on the helical content may be developed into another useful method for the estimate of the helical content of proteins. All of the four methods mentioned earlier have been applied to a number of proteins in aqueous solutions and the results are shown in Table 1. The good agreement among the four methods was indeed more than expected in view of the many assumptions involved, although it should also be pointed out that not all the four methods are entirely independent. Exceptions would certainly be found as more optical rotatory properties of proteins are studied. Indeed, Jirgensons[50] has recently proposed to classify proteins into three groups (see, also, this symposium, p. 166). Most proteins show a decrease in λ_c upon denaturation, which agrees with the prediction of our proposal. A second group includes those proteins the λ_c values of which vary little and the third group actually shows a rise in λ_c upon denaturation. Evidently these observations demonstrate the complexity of the problem and the oversimplification of our working hypothesis.

[48] C. Cohen and A. Szent-Györgyi have also pointed out that b_0 can be used as a measure of helical content. See *J. Amer. Chem. Soc.* **79**, 248 (1957).
[49] E. R. Blout and L. Stryer, *Proc. Natl. Acad. Sci.* **45**, 1591 (1959).
[50] B. Jirgensons and L. Straumanis, *Arch. Biochem. Biophys.* **68**, 319 (1957); B. Jirgensons, *Ibid.* **71**, 148 (1957); *Ibid.* **74**, 57, 70 (1958); *Ibid.* **78**, 227, 235 (1958); *Ibid.* **85**, 89, 632 (1959).

A discussion of some of the assumptions of our proposal seems in order. First the optical rotations of both the helical backbone and residues are postulated to be additive as if they were two distinct entities and each contribution were unaffected by the presence of the other. Strictly speaking, this can not be true on theoretical grounds since one is neglecting the contributions due to the interaction among side chains and also between side chains and helix. In fact these interactions can be quite large if the side chains and peptide groups are in close proximity. However, some cancellation would be expected in the case of most proteins because of the diversity of residues and the resultant effect might well be secondary to the total rotation. Secondly, the non-helical regions of the proteins must be arranged in such an irregular, non-periodical manner that their contributions to the optical rotation are effectively considered to be the same as those of the random coils, although they need not have the same flexibility as the latter. This again is debatable since the intrinsic residue rotations even of the coils are known to vary with their degrees of flexibility. In this sense the estimation of the helical content from the dispersion data rather than $[\alpha]_D$ or λ_c alone might be more reliable, since both a_0^H and b_0 of the helices are presumably less affected by the flexibility of the non-helical portions. (However, the a_0^H value cannot be accurately determined if the a_0^R value is uncertain.) Thirdly, our calculations assume that the helices have but one screw sense, probably right-handed. Conceivably, a mixture of equal amounts of right- and left-handed helices, if present, would approximately cancel out the helical rotations and exhibit a dispersion behavior similar to that of a random coil. The total rotation then measures the net excess of the dominant form plus the intrinsic residues, which clearly would result in an underestimate of the helical content. Although such mixed helices have not been observed in synthetic polypeptides, the possibility of their existence in proteins cannot be ruled out. A case in point is the A-chain of insulin which can not be completely folded into a one-handed helix due to the constraints of an intrachain disulfide bond.[51,52] Fourthly, the calibration curve was based on one synthetic polypeptide. The "abnormal" behavior of the Group II polypeptides would naturally pose a serious question about the validity of our proposal as applied to proteins in general. However, as mentioned earlier, it is doubtful that many of these residues would be localized extensively in a sequential array similar to that in a synthetic polypeptide and the possibility for them to form an excitation band of their own seems rather remote. Because of the diversity of these residues our values of a_0^H, b_0 and λ_0 may very probably approximate the average values for most proteins. Fifthly, the calibration curve for a high molecular weight polypeptide may not be directly applicable to very short helical chains. However, the recent work by Zimm *et al.*[53] has indicated that the optical rotation per unit length of helix appears to be essentially independent of length for any such helix with more than one turn, thus apparently disposing of this possible difficulty in the interpretation of the helicl content. Finally, the most serious assumption seems to be the neglect of the structural elements other than α-helix which may exist in proteins. For example, it is not unlikely that some regions of the protein molecule may be arranged in one of the β-structures, the contribution of which would by no means simulate that of the random coils. At present very little is known about these structural

[51] H. Lindley and R. S. Rollett, *Biochem. et Biophys. Acta* **18**, 183 (1955).
[52] B. W. Low, in *Currents in Biochemical Research* (Edited by D. E. Green) p. 422. Interscience, New York (1956).
[53] B. H. Zimm, P. Doty and K. Iso, *Proc. Natl. Acad. Sci.* **45**, 1601 (1959).

elements (see, however, the section on the intermolecular-hydrogen bonded form). In spite of all these reservations we feel confident, however, that our working hypothesis offers a semiquantitative estimate of the helical content in proteins. Our results seem also to confirm the existence of α-helical regions in native proteins. Of course one can always argue that proteins may contain other helical structures such as Pauling and Corey's γ-helix[8] or Low's π-helix[54] or even other more complicated structures which are energetically not much less favorable than the α-helix. We should nevertheless concede to reality and take full advantage of the existing knowledge at our disposal. Unless the future investigations point to an entirely different conclusion about the helical structure, we may feel sure that the α-helix is a predominant, though by no means unique, one in proteins. At the present stage our proposal may sharpen our understanding of the protein structure and serve as a framework of reference for at least its tentative interpretation. Just as the characterization of size and shape of macromolecules depends upon a combination of physical methods, so does the study of the internal structure of proteins. It is always dangerous to rely on a single technique. There is an urgent need for the development of independent methods, which combined with the rotatory dispersion study can give us a more reliable estimate of the helical content of proteins. Precise measurements of the deuterium-hydrogen exchange rates of proteins[55] might be one answer, although the estimates may be too high because of the possible presence of hydrogen bonds among side chains. The identification of an infrared band at 1640 cm^{-1} with the non-helical residues by Doty and Imahori[56] may offer another estimate of the helical content, although much work still has to be done along this line. Complicated as the protein molecules are, we should nevertheless not be too pessimistic about using optical rotatory dispersion. Numerous exceptions will no doubt be found as more experimental data are piled up. But with these new findings perhaps will come some modifications and improvements of our proposal. It is not inconceivable that as a result of future developments a better proposal will come forward.

Helix-coil transition in solutions

Synthetic polypeptides. Much of this subject has already been reviewed recently[5,57] and more details of our early work will be published in a forthcoming paper.[58] Suffice it to say, the helix-coil transition of polypeptides in solutions has been observed both as a function of solvent composition and temperature. In our first experiment the specific rotations of a high molecular weight poly-γ-benzyl-L-glutamate (PBLG) were examined in the mixtures of ethylene dichloride (EDC) and dichloroacetic acid (DCA).[19] The results as shown in Fig. 5 clearly demonstrate a first order transition in the vicinity of 76 volume per cent dichloroacetic acid, a coil-promoting solvent. (The small change upon the first addition of DCA was believed to be due to a change in the mean orientation of the benzyl glutamate groups relative to the helix core.) Such a sharp, reversible transition was also observed in other pairs of "helical" and "coiled" solvents. It

[54] B. W. Low and R. B. Baybutt, *J. Amer. Chem. Soc.* **74,** 5806 (1952); B. W. Low and H. J. Grenville-Wells *Proc. Natl. Acad. Sci.* **39,** 785 (1953).
[55] A. Hvidt, G. Johansen, K. Linderstrøm-Lang and F. Vaslow, *Compt. Rend. Trav. Lab. Carlsberg, Sér. Chim.* **29,** 129 (1954); I. M. Krause and K. Linderstrøm-Lang, *Ibid.* **29,** 385 (1955); K. Linderstrøm-Lang, Special Publication No. 2 of the Chemical Society, London (1955).
[56] P. Doty and K. Imahori, unpublished work.[2a]
[57] H. A. Scheraga, *Ann. Rev. Phys. Chem.* **10,** 191 (1959).
[58] P. Doty and J. T. Yang, manuscript in preparation.[2a,66]

could even be brought about by adding a non-solvent to DCA; for example, it took only about 3·4 volume per cent water to convert the coiled form into the α-helix and with more than 10 volume per cent water the polypeptide began to precipitate.[58] These rotation results were also fully supported by hydrodynamic measuremens such as intrinsic viscosity and flow birefringence.

Next, by studying the temperature dependence of the specific rotations at the center of the transition in Fig. 5, the coiled form was found to transform into the α-helical conformation over a relatively narrow temperature range for the high molecular weight

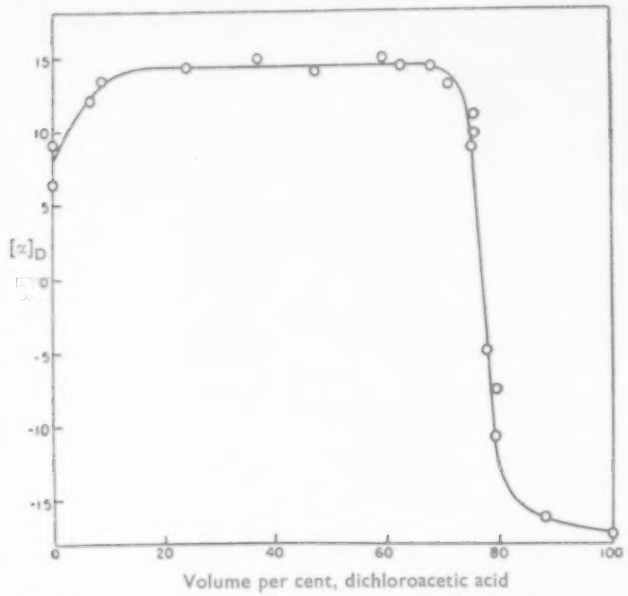

FIG. 5. Specific rotations (Na D line) of poly-γ-benzyl-L-glutamate (Mw = 350,000) in ethylene dichloride-dichloroacetic acid at 20°C.

sample (Fig. 6). However, as would be expected, the sharpness of this helix-coil transition was dependent on the molecular weight and molecular weight distribution. Of more interest was the fact that the helical conformation in this case was the stable form at higher temperatures, which was just the opposite of what has been observed with proteins. This transition was more strikingly demonstrated with the dispersion behavior (Fig. 7) which was normal for the randomly coiled conformation and anomalous for the helix in full agreement with the earlier conclusions. The reasons for the "unnatural" helix-coil transition have already been explained from thermodynamic considerations.[19] In passing it can be mentioned that after Schellman's thermodynamic treatment[59] of the stability of α-helices several elegant statistical mechanical theories[60–64] have recently been developed and applied to the same problem, all of which are in full support of our findings. Experimentally, a decrease of 11° in the

[59] J. A. Schellman, *Compt. Rend. Trav. Lab. Carlsberg, Sér. Chim.* **29**, 223, 230 (1955).
[60] B. H. Zimm and J. K. Bragg, *J. Chem. Phys.* **28**, 1246 (1958); **31**, 526 (1959).
[61] J. H. Gibbs and E. A. DiMarzio, *J. Chem. Phys.* **28**, 1247 (1958); **30**, 271 (1959).
[62] S. A. Rice, A. Wada and E. P. Geiduschek, *Disc. Faraday Soc.* **25**, 130 (1958).
[63] T. L. Hill, *J. Chem. Phys.* **30**, 383 (1959).
[64] L. Peller, *J. Phys. Chem.* **63**, 1194 (1959).

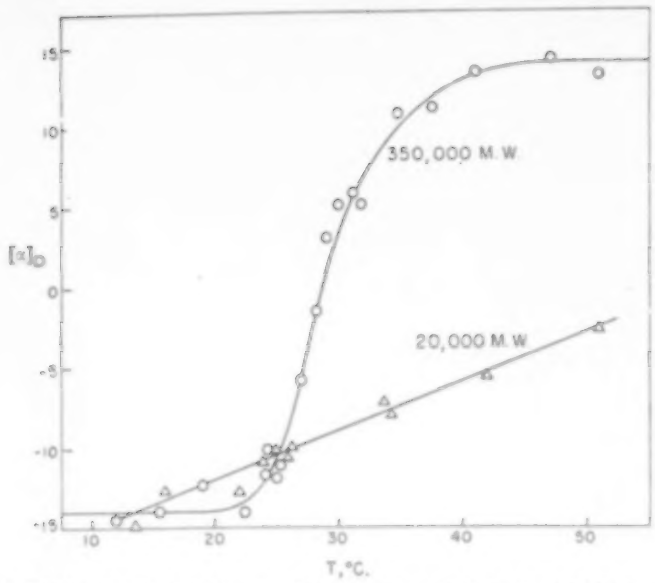

FIG. 6. The temperature dependence of the specific rotations (Na D line) for two poly-γ-benzyl L-glutamates in 20:80 (by weight) [or 34:76 (by volume)] ethylene dichloride-dichloroacetic acid.

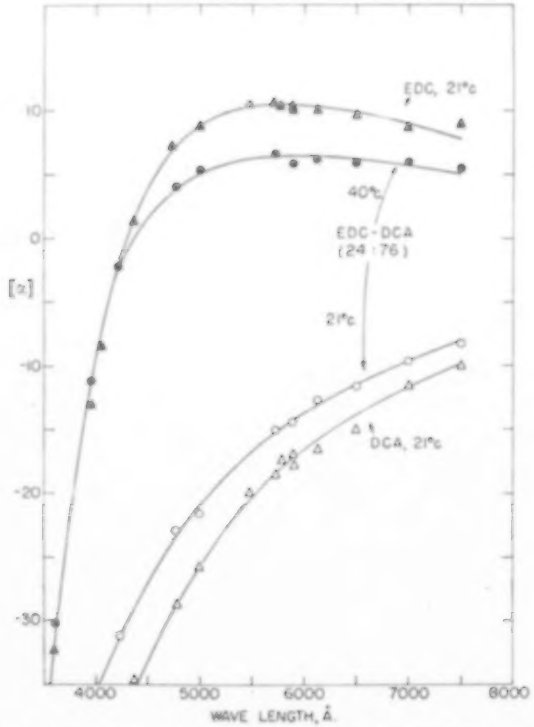

FIG. 7. Optical rotatory dispersion of poly-γ-benzyl-L-glutamate (Mw = 130,000) in two conformations. Anomalous dispersion: helical; normal dispersion: randomly coiled.

transition temperature as shown in Fig. 5 has also been reported when both the polypeptide and solvents were deuterated, although the magnitude and direction of this shift can not be predicted from *a priori* considerations as it depends upon the relative strengths of the various types of hydrogen bonds.[65]

Following the PBLG work, we then proceeded to study the helical stability of poly-α, L-glutamic acid (PLGA) in aqueous solutions, where pH and ionic strength become the important factors because of the repulsive forces arising from the ionized carboxyl group. The specific rotations and intrinsic viscosities in Fig. 8 again demonstrate a reversible transition over a relatively narrow pH range. Evidence also comes

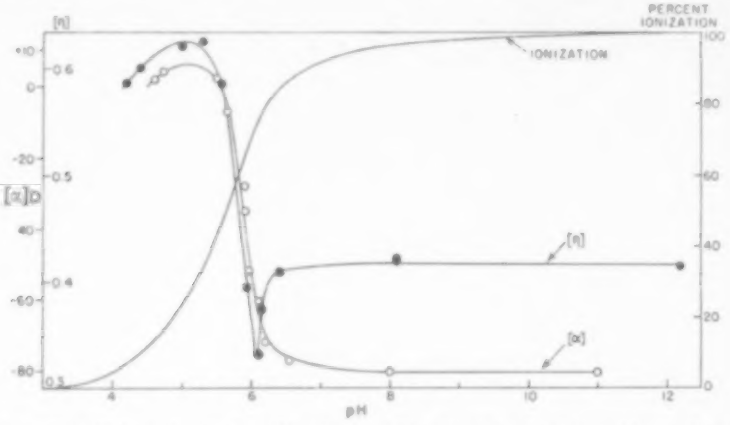

FIG. 8. The pH dependence of specific rotations (Na D line), intrinsic viscosities, and degrees of ionization of poly-α,L-glutamic acid (Mw = 34,000) in 0·2 M NaCl-dioxane (2:1 by volume) at 20°C.

from the rotatory dispersion behavior (Fig. 2) which clearly indicates that the helical conformation is the stable form at low pH, where the degree of ionization and thereby the electrostatic repulsion are small. The specific rotations of the coiled form at high pH were also found to be strongly dependent on the amount of salts added, and became less negative with higher ionic strength.[58] It should be pointed out that in the particular case of PLGA the unionized COOH groups at low pH might be hydrogen-bonded pairwise, thus contributing additional stability to the helical conformation. This perhaps also explained the unusual stability of the helical PLGA against the common denaturing agents such as urea and guanidine salts, which were found to shift the transition slightly toward the lower pH.[58]

The temperature dependence of the specific rotations of PLGA (Fig. 9) was quite different from that of PBLG and resembled a typical protein denaturation, the coiled conformation being more stable with increasing temperature. The broadening of the helix-coil transition could be attributed to many factors among which were the broad molecular weight distribution, the decreased ionization of the carboxyl groups with increasing temperature, etc. However, even with these explanations it poses a serious problem when one considers the protein denaturation. From theoretical calculations, Zimm *et al.*[53] have concluded that short helices show very broad transitions. It is therefore difficult to explain the sharp temperature dependence of the denaturation of

[65] M. Calvin, J. Hermans, Jr. and H. A. Scheraga, *J. Amer. Chem. Soc.* **81**, 5048 (1959).

proteins, since the helical regions in globular proteins must be quite short. These authors have suggested that the various helical regions of the protein molecule may be coupled in some way, thus sharpening the transition. It may even be possible that the tertiary structure (non-helical) of the native proteins is "frozen" in some way and undergoes transition simultaneously with the "melting" of the helices.

Proteins. Having found that polypeptide conformation in solutions depends primarily on the hydrogen-bonding ability of the solvents used, we then thought to

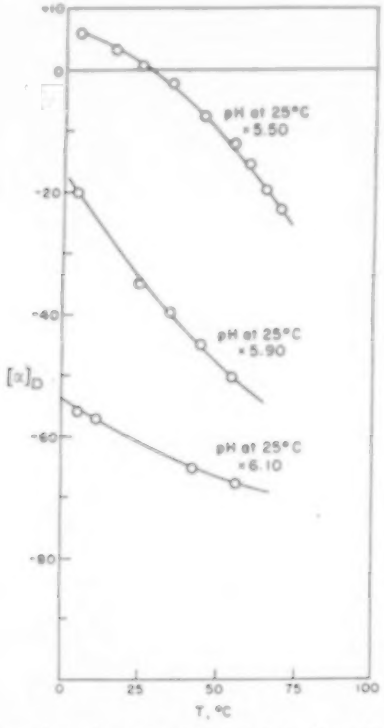

FIG. 9. The temperature dependence of specific rotations (Na D line) of poly-L,L-glutamic acid (Mw = 34,000) in 0·2 M NaCl-dioxane (2:1 by volume).

increase the helical contents in proteins by turning to non-aqueous solvents. Since most of the helical solvents for the polypeptides do not dissolve the proteins, we adopted in our early work an alternative of dissolving the proteins in a "coiled" solvent such as DCA and then adding as much as possible of a "helical" solvent such as EDC. In this way we were able to bring about a "reverse denaturation" and raise the helical contents above those found in aqueous solutions under usual conditions. For example, silk fibroin which exists as β-form in the solid state can be brought into the randomly coiled conformation in DCA and then converted into the predominantly helical conformation with the introduction of EDC (Fig. 10). (The magnitude of the specific rotations was due simply to the high glycine content of this protein [44 mole per cent] with consequent lower average residue weight than that of most proteins.) Additional evidence for this helix-coil transition also came from the dispersion measurements which indeed demonstrated a continuous change from the normal type for the coiled form into

the anomalous type for the helical conformation upon passing from DCA to 85 volume per cent EDC (Fig. 11).

The specific rotations of insulin as also shown in Fig. 10 appeared to display the same kind of helix-coil transition, although a full development of the helical conformation in this case was not realized as evidenced by the negative rotations even in the vicinity of 100% EDC. However, by performic acid oxidation and fractionation the

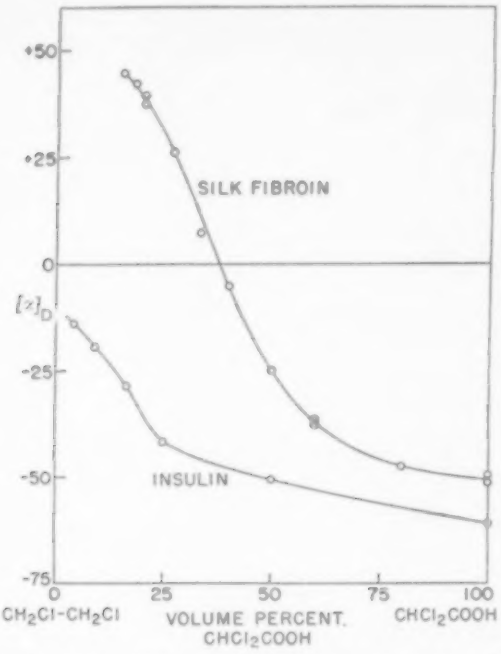

FIG. 10. Specific rotations (Na D line) of silk fibroin and insulin in ethylene dichloride–dichloroacetic acid at 20°C.

dispersion behavior of the B-chain of insulin in dimethyl formamide, a "helical" solvent, did closely resemble that of the synthetic polypeptide, thus indicating that at least the B-chain can exist as α-helix having the same screw sense as the polypeptide.[13] Both silk fibroin and insulin showed a gradual transition, probably because of the effects of heterogeneity of composition on the unfolding process. In the case of insulin, the smaller chain lengths also tended further to broaden this transition.

Similar studies were also made on ribonuclease and bovine serum albumin[13] which again showed the same kind of transition and the helical development of which could somewhat be aided by breaking the intrachain disulfide linkages through performic acid oxidation. The results of the oxidized proteins must, however, be considered preliminary since we have not carried out either here or in the case of the B-chain of insulin a characterization of the oxidized products employed. Hence the extent of oxidation and the degree of purification achieved were uncertain but these did not affect the conclusions reached here and above. These early studies also prompted a search for a better helical solvent which can dissolve many proteins and at the same

time is miscible with water. Imahori *et al.*[47] finally decided upon the use of 2-chloroethanol. The addition of this solvent to aqueous solutions indeed increased the helical content as measured by rotatory dipersion in nearly every case. Thus we have now at our disposal a wide range of solvents to be used for the studies of protein conformations and conformational changes.

Intermolecular-hydrogen bonded form

So far our discussions have been limited to the α-helical and randomly coiled conformations. Other polypeptide structures are by no means unimportant, even though

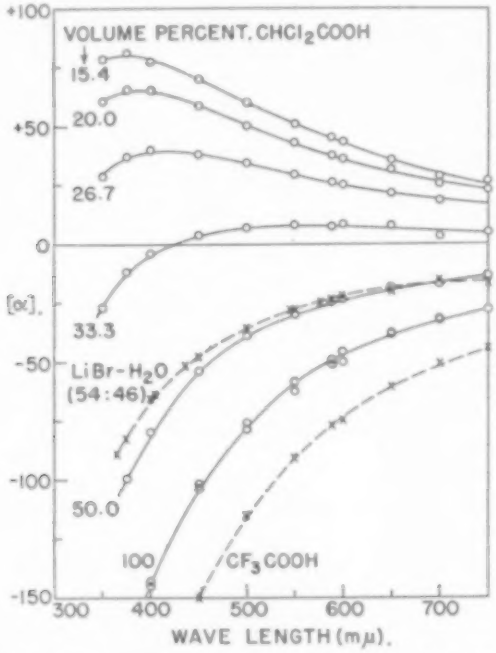

FIG. 11. Optical rotatory dispersion of silk fibroin in ethylene dichloride–dichloroacetic acid at 20°C. The two broken lines represent the data in 54:46 (by weight) lithium bromide–water and in trifluoroacetic acid respectively.

very little information has as yet been available. Indeed our analysis of the dispersion behavior of proteins would be incomplete without knowing the contributions and complications due to these important structures. One such structure which has received considerable attention is the intermolecuar-hydrogen bonded form, commonly called the β-form. Infrared spectral studies[66,67] first demonstrated that polypeptides of sufficiently low molecular weight did not form an α-helical conformation. Instead, they existed in an intermolecularly hydrogen bonded form, an aggregated state similar to the β-form known in the solid state. From thermodynamic considerations Schellman[59] has also reached the conclusion that the α-helices are only stable above a certain critical length which depends on a number of factors such as the composition of the polypeptide, the environment and the temperature. Dispersion measurements were

[66] E. G. Ambrose and A. Elliott, *Proc. Roy. Soc.* A **205**, 47 (1951).
[67] E. R. Blout and A. Asadourian, *J. Amer. Chem. Soc.* **78**, 955 (1956).

therefore made on a low molecular weight PBLG sample in chloroform, chloroform saturated with formamide, and dichloroacetic acid, the results of which are shown in Fig. 12. Unlike the α-helical polypeptide the rotations of the β-form were concentration-dependent. At high concentrations in a poor solvent (chloroform in this case) they were positive and showed normal dispersion behavior. At low concentrations they became negative, similar to those in the randomly coiled form (in DCA), a fact

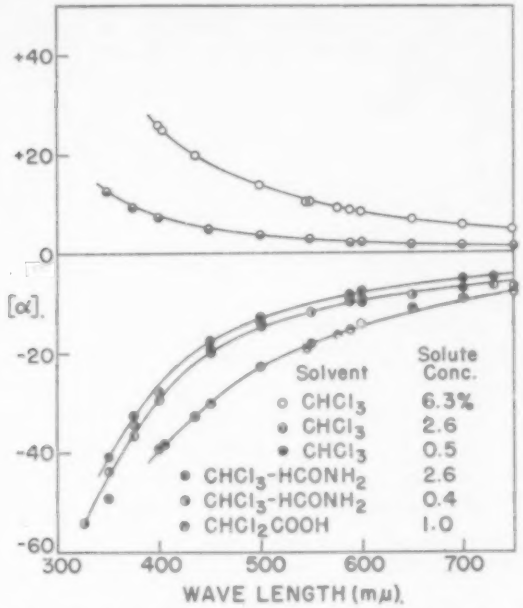

FIG. 12. Optical rotatory dispersion of very low molecular weight poly-γ-benzyl-L-glutamate in several solvents at different concentrations at 20°C.

perhaps indicative of the disruption of these aggregates. This β-form was also unstable when small amounts of more strongly hydrogen-bonding agent such as formamide was introduced.

No rotation studies seem to have been reported that demonstrate the existence of the β-form in protein solutions. It seems very suggestive to think that such conformation, if present in the protein solutions, will probably also show a positive contribution as contrasted to the negative intrinsic rotation of the L-residues, although the observations in organic solvents may be quite different from those in aqueous solutions. The fact that the β-form is strongly concentration-dependent and unstable in the presence of traces of strongly hydrogen-bonding agents prevents us from making any further speculation. Very recently, however, Imahori[68] has demonstrated that the β-form of proteins in aqueous solutions does indeed make a positive contribution to the rotation. First he found that our data in Fig. 12 could equally well be fitted with equations (4) and (5) for the α-helices, even though this again seems purely empirical. The interesting point was that both a_0^H (here for the sake of simplicity the same symbol was retained for the β-form, although the superscript H originally referred to the helix) and b_0 were found to be positive with λ_0 presumably set at 0·212 μ, as contrasted

[68] K. Imahori, *J. Mol. Biol.* in press.

with the opposite signs found for the α-helices. Thus at least empirically Imahori's suggestion offers a means of distinguishing these two conformations, provided that future experiments support the correctness of his interpretation. By making the dispersion measurements of the aggregates of denatured pepsin and bovine serum albumin (in the presence of salt to prevent precipitation, ionic strength not given), Imahori found that the $(\lambda^2 - \lambda_0^2)[\alpha]_\lambda - 1/(\lambda^2 - \lambda_0^2)$ plot [equation (4)] of both proteins did give a positive slope, although the total rotations were negative throughout the range of wavelengths studied. Take, for eaxample, the β-form of bovine serum albumin, which gave $a_0^{II} = +250$ and $b_0 = +234$, as contrasted with +650 and −630 for the pure α-helix. If one accepts this interpretation and pursues it further, an interesting feature immediately follows. The dispersion plot, $(\lambda^2 - \lambda_0^2)[\alpha]_\lambda$ versus $1/(\lambda^2 - \lambda_0^2)$ according to equation (4), for a mixture of the α-helices and β-forms would give a smaller slope (in magnitude) than it would have been in the absence of the β-form. Consequently the helical content based on the b_0 value would be underestimated. Likewise, the apparent a_0^{II} value would be smaller than the true value for the α-helix, thus again yielding a lower estimate of the helical content. On the other hand, the estimates from the two methods should be different since the a_0^{II} and b_0 constants are both positive for the β-form but have opposite sign for the α-helix. For instance, a mixture of 50 per cent α-helix and 50 β-form gives an apparent helical content of 69 per cent from the a_0^{II} method and 32 per cent from the b_0 method respectively. Again, let us take native bovine serum albumin for an illustration, for which Imahori has listed $a_0^{II} = +291$ and $b_0 = -170$. Thus for a mixture of 58 per cent native protein and 42 per cent β-form, the apparent b_0 value becomes zero and $a_0^{II} = 274$, which correspond to zero and 42 per cent helical content from the two methods. Also of interest to note is the fact that the $[\alpha]_D$ value, about −60°, would remain virtually the same for this mixture as for the native protein, but the $\lambda^2[\alpha]_\lambda$ versus $[\alpha]_\lambda$ plot according to equation (2) would give a λ_c of 0·212 μ, which amounts to 100 per cent random coils, although the $[\alpha]_D$ value indicates otherwise. Further, let us consider some denaturation process which may disintegrate the aggregated β-form but not break the α-helices. This would result in an increase in the magnitude of the b_0 value and thus the estimate of the helical content in our terminology. Or to put it in another way, the $\lambda^2[\alpha]_\lambda - [\alpha]_\lambda$ plot would yield a higher λ_c value during this process. It may also not be inconceivable if, in addition to the disruption of the β-form, the helices also undergo partial unfolding. Through cancellation of the two contributions the apparent λ_c value may remain virtually unchanged. Since our interpretation is confined to the intermolecularly hydrogen bonded form, it is, of course, very difficult to predict whether these discussions have any bearing on the recent findings by Jirgensons[50]. In this respect it is interesting to mention Jirgensons' observation that high amounts of hydroxyamino acids are usually found in proteins having abnormally low dispersion constants, e.g., the γ-globulins, Bence-Jones proteins and pepsin. These hydroxyl groups can of course form intermolecular as well as intramolecular hydrogen bonds among themselves or with some other groups. Little is known, however, about the internal structure of proteins. We must await further investigations before making a more intelligent guess. There is every reason to believe that this goal can eventually be achieved by a combination of physical and chemical methods at our disposal. We also feel confident that the optical rotatory dispersion method will play an even more useful role than it now does, as our knowledge of protein structure advances.

Acknowledgments—The author is indebted to Professor P. Doty for many invaluable suggestions and for providing much of the late information included in this paper. The author's early work, as reported herein, was completed in Professor Doty's laboratory. The polypeptide samples employed were generously supplied by Dr. E. R. Blout to whom the author also owes many interesting discussions.

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OPTICAL ROTATORY DISPERSION OF GLOBULAR PROTEINS*

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INTRODUCTION

THE optical rotatory dispersion of globular proteins has been studied recently in several laboratories.[1–9]. This interest is due chiefly to the possibility to correlate the optical rotatory dispersion data with the structure and configuration† of the macromolecules, and good reviews are available on the latter subject.[10–12] The purpose of this paper is to present new results on several proteins, as well as to tabulate the earlier results. A short discussion will be added, indicating the insufficiency of the presently dominating helix-coil transitions model for the treatment of these phenomena.

EXPERIMENTAL METHODS

Most of the 42 proteins mentioned in this paper have been described in previous publications of this and other authors.[1–9,13,14] The hormones prolactin and somatotropin (growth hormone) were gifts from Professor C. H. Li of University of California. The lima bean trypsin inhibitors I and II were isolated in our Laboratory by Dr. T. Ikenaka from a commercial product by chromatographic fractionation, and a detailed report on the inhibitors will be published elsewhere. Ovomucoid was purchased from Pentex, and appeared to be a pure protein containing 9·9 per cent hexose and 15 per cent hexosamine; the protein appeared homogeneous in sedimentation, and it exhibited one slightly asymmetric peak in free boundary electrophoresis. The luteinizing hormone LH_2 was a gift from Dr. D. N. Ward of our Biochemistry Department; a detailed study on the chemistry of this protein will be published by Dr. Ward in due time. The macroglobulins and myeloma globulins were isolated from the blood

* This study was supported in part by grants from the National Cancer Institute, National Institutes of Health, grant NCI-1785, the Robert A. Welch Foundation, Houston, Texas, grant G-051, and the American Cancer Society (institutional grants).

† Dr. E. R. Blout suggests to use the term "conformation" instead of "configuration", if this concerns the long polypeptide chains.

[1] B. Jirgensons, *Arch. Biochem. Biophys.* **78**, 235 (1958).
[2] B. Jirgensons, *Arch. Biochem. Biophys.* **74**, 57 (1958).
[3] B. Jirgensons, *Arch. Biochem. Biophys.* **85**, 532 (1959).
[4] A. Maeda and A. Oikawa, *J. Biochem.* **46**, 495 (1959).
[5] J. A. Schellman, *Compt. Rend. Trav. Lab. Carlsberg* **30**, 415 (1958).
[6] J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957).
[7] B. Jirgensons, *Arch. Biochem. Biophys.* **74**, 70 (1958).
[8] B. Jirgensons, *Arch. Biochem. Biophys.* **85**, 89 (1959).
[9] G. E. Perlmann, *Proc. Natl. Acad. Sci.* **45**, 915 (1959).
[10] C. Schellman and J. A. Schellman, *Compt. Rend. Trav. Lab. Carlsberg* **30**, 463 (1958).
[11] W. Kauzmann, *Ann. Rev. Phys. Chem.* **8**, 413 (1957); *Advanc. Protein Chem.* **14**, 1 (1959).
[12] S. J. Leach, *Rev. Pure Appl. Chem., Austr.* **9**, 33 (1959).
[13] B. Jirgensons and T. Ikenaka, *Makromol. Chem.* **31**, 112 (1959).
[14] W. F. Harrington and M. Sela, *Biochem. Biophys. Acta* **31**, 427 (1959); C. B. Anfinsen in *Symposium on Protein Structure* (Edited by A. Neuberger) p. 223. John Wiley, New York; Methuen, London (1958).

of patients in this and other laboratories; some of the macroglobulins were gifts from Dr. H. F. Deutsch (University of Wisconsin), and more detailed reports on the macroglobulins and myeloma globulins are in preparation.

The proteins were dissolved in water or in aqueous salt solutions or alkalies of low or moderate ionic strength (usually 0·05–0·1). As shown in several previous publications,[1,2] such slight variation of ionic strength does not affect the rotatory properties. (Somewhat exceptional in this respect are some of the redox enzymes, such as aldolase). The pH of the solutions of the native proteins was rather close to the isoelectric point in each case avoiding extreme acid or alkaline reaction. Generally, the native proteins were studied under such conditions when they are maximally stable and yield clear solutions. In some of the denaturation experiments, the solutions were either strongly acid (pH 1·5–2·5) or strongly alkaline (pH 11–12·0), although heating was avoided. Denaturation with guanidine thiocyanate, however, was accomplished by warming the solutions at 50°C for 1–1½ hours. The guanidine thiocyanate was a product of Eastman Coroporation; it was recrystallized from hot methanol. The protein concentration was usually between 0·5–4 per cent, and it was determined either by gravimetric method[1,2] or by micro-Kjeldahl nitrogen analyses,[3] or by both methods for one and the same solution.

The optical rotation was measured with a Rudolph Model 80 photoelectric spectropolarimeter. A Hanovia mercury burner was used as the light source, and the lines at 578, 546·1, 435·8, 404·7, 390·6, 366·3, 365·0, and 334·1 mμ were used in most instances; a sodium lamp, and a zirconium white light source also were used in some experiments.[2] The measurements were made at room temperature of 24–26° ($\pm$2) in 10, 20, or 40 cm semi-micro tubes with glass windows. The latter were fastened with the minimum of stress, and only such windows (end plates) were used that showed a positional variation of the zero point not larger than $\pm$0·02° of the α. The zero points of the tubes, and the instrument without tube, was checked frequently. A quartz control plate certified by the National Bureau of Standards (1614—NBS—1958) also was occasionally used for checking the performance of the instrument.

The dispersion constants were evaluated according to the Yang–Doty method, viz. by plotting $\lambda^2 . [\alpha]$ versus $[\alpha]$. The $[\alpha]_D$ values were calculated from the one-term modified Drude equation. The errors in the dispersion constants λ_0 (or λ_c) were found to be $\pm$2–3 mμ in most cases, while the accuracy in the $[\alpha]_D$ values is in the range of 0·5–2°. This is the reproducibility as obtained with blameless preparations in repeated series of measurements. However, it must be pointed out that the differences in the specific rotation and dispersion constant values found on different preparations of same protein may be much larger than those mentioned. This may be due to either impurities or partial denaturation of the protein. Especially great are the effects produced by colored impurities, e.g. a specimen of impure pancreatic α-amylase[7] yielded a dispersion constant about 30 mμ lower than a colorless pure preparation.[3] Because of the same reason, the results obtained with the not quite colorless preparations of alcohol dehydrogenase and glyceraldehyde phosphate dehydrogenase probably involve larger errors. Less serious sources of error are those associated with defects in the technique of measurement. If the optical activity is low, a considerable error may be caused by the stresses in the tube windows. Other errors may be caused by the weakness and instability of the light source. If a white light source is used, such as zirconium lamp, a considerable error may be caused by the necessity to work with too wide slit.

Results

The results are compiled in Tables 1–4. With respect to the values of the dispersion constants (λ_0 or λ_c), the globular proteins can be classified into three groups (7):

(1) proteins with relatively high dispersion constants (Table 1),
(2) proteins with relatively low dispersion constants (Table 2),
(3) proteins with abnormally low dispersion constants (Table 3).

TABLE 1. ROTATORY DISPERSION AND SPECIFIC ROTATION OF NATIVE GLOBULAR PROTEINS
Class I: proteins with *high* λ_0 (λ_0) of 250–280 mμ.

	λ_0	K*	$-[\alpha]_D$	Reference
1. Albumin, egg	266	8·3	30°	1
2. Albumin, serum	267	17·4	63	2,6
3. Albumin, mercapt.	266	18·0	65	2
4. Aldolase	277	7·5	28	3
5. Amylase, bacterial	261	7·2	26	1
6. Amylase, β	277	7·0	26	3
7. Amylase, taka	271	3·3	12	3
	293	3·1	12	4
8. Carboxypeptidase	269	5·7	21	3
9. Dehydrogenase, alcohol†	248	8·7	30	3
10. Dehydrogenase lactic acid	264	11·8	43	3
11. Ficin	261	11·2	40	3
12. α-Glycoprotein, acid	249	6·5	23	2
13. Insulin	267	8·5	31	5
14. Lysozyme	254	13·5	47	6,7
15. Prolactin	276	10·0	37	14*a*
16. Somatotropin	277	7·7	29	14*a*
17. Trypsin inhibitor, lima bean, I	258	7·6	27	14*b*
18. Trypsin inhibitor, lima bean, II	280	4·7	17	14*b*

* If the λ is expressed in mμ, the actual values of K can be obtained by multiplying the K-values of the Tables 1–4 by 10^{-6}.

† According to a recent report of H. Sund [*Biochem. Z.* **333**, 205 (1960)], the λ_c (viz. λ_0 according to the notation used here) of pure and enzymically very active alcohol dehydrogenase is 262 mμ.

It has been found that the high λ_0-values of the proteins of Table 1 decrease upon denaturation (unfolding) considerably. At the same time the specific rotation becomes more negative. The proteins of Tables 2 and 3, on the contrary, show a much smaller change in both λ_0 and $[\alpha]_D$ upon denaturation. The protins of Table 3 either retain their low λ_0-values on denaturation or even show an increase of the constants on unfolding, while the specific rotation becomes more negative. Although the Bence–Jones proteins, γ-globulins, and macroglobulins exhibit a considerable individuality, the dispersion constants of all of them were found in the extremely low range of 180–215 mμ, and they increase on denaturation. The rotatory properties of the denatured globular proteins are compiled in Table 4. Denaturation was ascertained either by the loss of solubility upon removal of the denaturing agent or by the loss of enzymic

[14a] B. Jirgensons, *Arch. Biochem. Biophys.* **91**, 123 (1960).
[14b] B. Jirgensons, T. Ikenaka and V. Gorguraki, *Makromol. Chem.* **39**, 149 (1960).

TABLE 2. ROTATORY DISPERSION AND SPECIFIC ROTATION OF NATIVE GLOBULAR PROTEINS
Class II: proteins with *low* λ_0 (λ_c) of 220–245 mμ.

	λ_0	K	$-[\alpha]_D$	Reference
1. Amylase, α, pancreatic	243	13·5	47°	3
2. α-Casein	223	26·9	90	2
3. α-Chymotrypsin	232	18·7	64	3, 5 (p. 455)
4. β-Chymotrypsin	234	18·4	63	7
5. γ-Chymotrypsin	237	18·3	63	7
6. Chymotrypsinogen	235	23·1	79	3, 5 (p. 450)
7. Dehydrogenase, glycerald. phosphate	238	17·3	60	3
8. Globulin, β, metal binding	242	13·3	46	2
9. Lactoglobulin, β	240	9·2	32	2, 5 (p. 396)
10. Ovomucoid	228	21·5	73	14*c*
11. Papain	231	18·5	63	3
12. Ribonuclease	231	21·0	71	5 (p. 458) 6, 7.
13. Trypsin	235	11·7	40	3
14. Trypsinogen	232	13·0	44	3

TABLE 3. ROTATORY DISPERSION AND SPECIFIC ROTATION OF NATIVE GLOBULAR PROTEINS
Class III: proteins with very low λ_0 of 180–220 mμ

	λ_0	K	$-[\alpha]_D$	References
1. Bence–Jones proteins*	180–210	11–17	37–55°	1,2, 8
2. β-Casein	218	42·1	140	2
3. γ-Globulin, normal*	200–217	14–16	44–53	1, 2, and unpublished
4. Luteinizing hormone, LH_2†	212	24·0	80	14*a*
5. Macroglobulin, serum*	196–205	8–10	30–38	14*c*
6. Mycloma globulins, serum*	206–218	11–13	40–45	14*c*
7. Pepsin	216	20·7	69	1, 3, 7, 9
8. Phosvitin‡	207–225	14–24	47–79	2, 7
9. Rennin	210	16·7	55	1
10. Trypsin inhibitor, soy bean	217	23·6	79	3,7, and unpublished

* Variations in the constants of Bence–Jones proteins, γ-Globulins, macroglobulins and myeloma globulins are due to individuality.

† This result was obtained with a 1·0% of sheep luteinizing hormone in acetate buffer of pH 4·7, and ionic strength of 0·05. An even lower λ_0 of 207 was obtained in weakly alkaline borate buffer of pH 8·2.

‡ Variations in the instance of phosvitin are caused by the changes in expansion and unfolding at various pH values.

activity or strong increase of viscosity, usually by two or three criteria, in addition to the changes in the rotatory behavior. 2 M guanidine thiocyanate at 50° appeared to be effective for most proteins, although in some instances 3–4 M guanidine thiocyanate produced a more complete denaturation than 2 M thiocyanate. It was not attempted to include in this Table all results published on this subject by various authors, but only the most definitive and characteristic data are included.

[14c] B. Jirgensons, *Arch. Biochem. Biophys.* **89**, 48 (1960).

The abnormal proteins of group III (Table 3) deserve special attention. Of the well defined crystallized proteins of this class, pepsin has been investigated to a great detail.[1,2,7,9] Five different specimens of one, two or three times recrystallized pepsin possessing full enzymic activity have been examined by the author of this paper. The concentrations of the protein and added neutral salts have been varied; the pH of the solutions was in the range of 4·8–5·5, and autodigestion was avoided by using

TABLE 4. ROTATORY DISPERSION AND SPECIFIC ROTATION OF *denatured* GLOBULAR PROTEINS

Protein	Denaturing agent	λ_0	K	$-[\alpha]_D$	References
1. Albumin, egg	Urea	226	27·5	93°	10
2. Albumin, serum	Urea	221	30·7	103	10
Albumin, serum	Guanidine-HCNS	225	27·0	91	13
Albumin, serum oxidized	Guanidine-HCNS	218	21·3	71	13
3. Aldolase	Alkali, pH 11·2	220	27·5	92	unpublished
4. Amylase, bacterial	Guanidine-HCNS	219	19·2	64	unpublished
5. Amylase, taka	Alkali	213	24·1	80	1
6. Dehydrogenase, lactic acid	Alkali, pH 11·2	225	25·3	95	unpublished
7. Insulin	Urea	226	24·9	84	10
8. Lysozyme	Guanidine-HCNS, 2 M	230	21·2	72	unpublished
Lysozyme	Guanidine-HCNS, 3 M	226	22·2	75	unpublished
Lysozyme	Guanidine-HCNS, 4 M	215	24·9	83	unpublished
9. Prolactin	Guanidine-HCNS, 4 M	207	23·4	77	unpublished
10. Somatotropin	Guanidine-HCNS, 4 M	221	21·3	71	unpublished
11. α-Chymotrypsin	Urea	220	31·6	106	10
α-Chymotrypsin	Guanisine-HCNS, 2 M	218	27·8	93	unpublished
12. Chymotrypsinogen	Urea	224	32·9	111	10
Chymotrypsinogen	Guanidine-HCNS, 2.6 M	209	36·0	119	unpublished
13. β-Lactoglobulin	Urea	225	32·9	111	10
14. Ribonuclease	Urea	220	30·7	103	10, 14
15. Trypsin	Guanidine-HCNS, 2 M	223	26·2	88	unpublished
16. Bence-Jones prot.	Acid or guanid.-HCNS	(218)	(24)		
17. Pepsin	Alkali	224	25·2	85	7
	Alkali, pH 8·9	223	27·5	92	unpublished
1.8 γ-Globulin	Acid or guanid.-HCNS	(220)	(26)	(87)	1, and unpubl.

fresh solutions. All such solutions yielded dispersion constants of 212–216 mμ. Denaturation by means of weak alkali (pH 7·8–9·6) or guanidine thiocyanate always yielded somewhat higher dispersion constants of 222–226 mμ (±2).[1,7] The low dispersion constant for native pepsin has been found also by Perlmann.[9] Also it has been found by Perlmann that the slight rotational changes that occur on long standing of mixtures containing guanidine hydrochloride are due to autodigestion.

The soy bean trypsin inhibitor is another example of proteins of class III that was very thoroughly investigated. Five different preparations of one to five times recrystallized inhibitor were used[1,7*] at various conditions at which the protein retained its inhibiting activity. Altogether 16 series of dispersion measurements have been run with this protein, and, since the solutions were colorless and clear, and the optical activity of the protein is high, the results can be considered as relatively very accurate.

* Unpublished results.

The dispersion constants obtained in these experiments varied within the narrow range of 210–219 mμ. The average value as computed from the best series of measurements (i.e. the one showing the least scatter in the linear plots λ^2. [α] versus [α]) was 217 mμ. The native state was ascertained not only by the enzymic inhibition tests but also by viscosity measurements. The intrinsic viscosity of this protein was found to be 0·050 dl/g at pH 7·8 and ionic strength of 0·05.[7]

The Bence–Jones proteins and the γ-globulins also have been studied to a considerable extent. Although none of them were crystallized preparations, the native state was ascertained by the low specific rotation values and low viscosities of the solutions.

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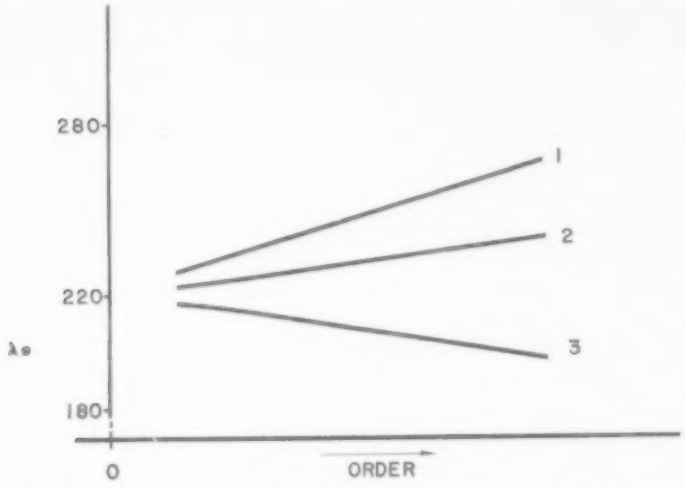

FIG. 1. A qualitative illustration of the dependence of the dispersion constants on the degree of disorganization of the macromolecules of proteins. Curve 1, proteins of group I; curve 2, proteins of group II; curve 3, proteins of group III.

Most of the solutions were quite colorless so that the readings could be extended into ultraviolet up to 365 or even 334 mμ lines of the mercury spectrum. The experimental error, as calculated by the usual statistical methods, in these series of measurements was between 2 and 5 mμ for the dispersion constant.

The changes of the dispersion constants on denaturation (i.e. expansion and disorganization) of the proteins of the three classes are illustrated in Fig. 1. Since a considerable variation is observed in each class, the curves actually should be drawn as bands. Some of the proteins of class III, such as pepsin, show only a slight change of the dispersion constant on denaturation, so that curve 3 shows this change in an exaggerated fashion.

Discussion

Conclusions. The results on the optical rotatory dispersion of native and denatured globular proteins can be summarized in the form of the following three statements:

(1) The one-term Drude rule holds for all colorless native and denatured proteins, when the rotatory dispersion is measured in the wave length range of 365–600 mμ;

(2) The dispersion constants of all *native* globular proteins are within the range of 180–280 mμ;

(3) The dispersion constants of all globular compact proteins converge to a common value of 210–220 mμ on denaturation (expansion, unfolding, disorganization) of the macromolecules.

Rotatory dispersion studies of other authors on polyaminoacids have indicated that the one-term Drude rule holds only for the solutions in which these macromolecules have a random-chain conformation.[6] Since both native and denatured proteins obey the one-term Drude law, one may be tempted to conclude that the macromolecules of native globular proteins do not possess a unique ordered (e.g. α-helical) conformation, but that they are disordered chains. Yang and Doty[6], who thoroughly studied this problem, came to the conclusion that only a relatively small core of the globular macromolecule is orderly folded in aqueous solution, and that this ordered (helical) portion can be increased by using certain non-aqueous solvents. These efforts to reconcile the rotatory dispersion results with a simple helix-coil transition model have been rather successful with several polyamino acids[6,15] but they have been less successful with proteins. For example, this reasoning led to the conclusion that the assumed helical cores must be of one definite screw sense, and that the dispersion constants must decrease on disorganization. In reality, however, several proteins of the class III show an increase of the dispersion constants on disorganization.

Since many of the native globular proteins possessing low dispersion constants have been crystallized (e.g. ribonuclease, pepsin, soy bean trypsin inhibitor), there is every reason to believe that their macromolecules are *ordered*, and that denaturation results in expansion, unfolding, and disorganization of the unique native conformation (or "configuration" in the more common terminology). However, the rotatory dispersion data indicate that these proteins have very little, if any, helical portions, and that their secondary and tertiary structure must differ from that of the proteins possessing high dispersion constants. Aside of the now popular α-helix, other types of secondary structure should be considered in globular proteins. Also the fact should be considered that, due to the interactions of the peptide side groups, the formation of a perfect helix may be hindered. The X-ray structural analysis of myoglobin has shown that the tertiary structure (folding of the chain into a compact body) is very complex;[16] the single polypeptide chain, whatever the secondary structure of it may be, is folded many times. Even if a helical secondary structure would be revealed in the straight sections of the chain, this cannot be the case at the many turns. The variety of the dispersion constants of the native globular proteins can be caused by some variety in the *secondary* as well as *tertiary* structure of the chains in the macromolecules of the proteins. Further studies, e.g. with a very high resolution X-ray diffraction, should show how helical the secondary structure of the polypeptide chain really is, and in what extent the tertiary structure differs in different globular proteins*.

Meantime, however, other attempts can be made in order to correlate the rotatory dispersion data with other properties of the proteins, such as the *amino acid composition* and *primary structure*. It is noteworthy that most of the proteins of class III, such as

* J. C. Kendrew, R. E. Dickerson, B. E. Strandberg, R. G. Hart, D. R. Davies, D. C. Phillips and V. C. Shore [*Nature, Lond.* **185**, 422 (1960)] indeed were able to show that there are helical structures in the macromolecules of crystalline myoglobulin.

[15] E. R. Blout, P. Doty and J. T. Yang, *J. Amer. Chem. Soc.* **79**, 749 (1957); M. Idelson and E. R. Blout, *Ibid.* **80**, 4631 (1958); P. Doty in *Proceedings of the Fourth International Congress of Biochemistry* Vol IX. Pergamon Press, London (1959); *Physical Chemistry of High Polymers of Biological Interest* (Edited by O. Kratky) p. 8. Pergamon Press, London, New York (1959).

[16] J. C. Kendrew, *Nature, Lond.* **182**, 764 (1958).

pepsin,[17] γ-globulin, and the Bence–Jones proteins, are rich in the hydroxyamino acids serine and threonine.[3,8] The possibility of interchain hydrogen bonds has already been considered, but covalent ester bonds between the hydroxyl groups and carboxyls of the chains also should be considered.[3,8] It is noteworthy that phosphodiester bonds have already been ascertained in pepsin.[18] In the structural considerations, thus far only the secondary folding of the chain has been considered, and the intrachain hydrogen bonds have been thought to be responsible for the helical folding. However, more and more evidence is accumulating that suggests the importance of other causes for the secondary and tertiary structure, such as electrostatic interaction between charged groups, hydrophoby of the hydrocarbon side groups, as well as the covalent intra- and inter-chain bonds.[11,19] The latter can affect the rotatory properties in two ways: (1) by affecting the asymmetry at the carbon atoms,[20] and (2) by affecting the folding of the chain. The second effect is probably the decisive, although the first should not be altogether neglected. The direct "short range" effects of the side groups, e.g. disulfide bonds, however, are difficult to estimate, since a chemical modification affects not only these groups but also the conformation of the whole chain or at least of some of its parts.[12,13]

The conclusion that the rotatory dispersion of globular proteins is determined by the conformation of the polypeptide chain is supported also by the finding that the dispersion constants of all denatured (unfolded, disorganized) globular proteins converge to the same value of 210–220 mμ. Hence the radicals attached to the asymmetric carbon atoms become unimportant for the numerical value of the dispersion constant (λ_0) when the chain is unfolded and disorganized. While the dispersion constants converge to a common number, the specific rotation of the disorganized proteins seems to depend not only on the degree of disorganization but also on the individual contributions of the asymmetry centers, i.e. the amino acid composition and primary structure of the proteins (see Table 4).

If the differences in the dispersion constants of the native globular proteins would be determined by the ratio of helical to random conformations, all the presumably disordered macromolecules, viz. those with the low λ_0 values, should have higher intrinsic viscosity constants than the presumably helical molecules with their high λ_0. (It is, of course, assumed that in any globular macromolecule the helix is folded thus yielding a relatively symmetrical corpuscular unit). This, however, is not so. The solutions of pepsin, soy bean trypsin inhibitor,[7] as well as those of the Bence–Jones proteins,[21] all possess about the same intrinsic viscosity values as any of the proteins of class I. For instance, the intrinsic viscosity of native pepsin at a moderate ionic strength of 0·01–0·10 is 0·030–0·035,[7,22] while that of serum albumin (class I) is 0·036–0·040 dl/g.[23] Of the proteins compiled in Tables 2 and 3, only the caseins and the macroglobulins have relatively more highly viscous solutions, while the visocity of the phosvitin is strongly dependent on the acidity of the solutions.[2,7]

[17] O. O. Blumenfeld and G. E. Perlmann, *J. Gen. Physiol.* **42**, 553 (1959).
[18] G. E. Perlmann, *J. Gen. Physiol.* **41**, 441 (1958).
[19] C. Tanford in *Symposium on Protein Structure* p. 35. John Wiley, New York; Methuen, London (1958).
[20] J. E. Turner, R. T. Bottle and F. Haurowitz, *J. Amer. Chem. Soc.* **80**, 4117 (1958).
[21] B. Jirgensons, A. J. Landua and J. Awapara, *Biochim. Biophys. Acta* **9**, 625 (1952); B. Jirgensons, *Arch. Biochem. Biophys.* **39**, 261 (1952); **48**, 154 (1954).
[22] H. Edelhoch, *J. Amer. Chem. Soc.* **79**, 6100 (1957).
[23] B. Jirgensons, *Makromol. Chem.* **16**, 192 (1955); C. Tanford and J. G. Buzzell, *J. Phys. Chem.* **60**, 225 (1956).

The results of this paper, moreover, indicate that a low dispersion constant alone is an insufficient characteristic for the state of conformation. In several instances, especially in the group III, the dispersion constants practically do notchange on denaturation, while the solubility, viscosity, specific rotation, and other properties change considerably. This was observed, for example, in the denaturation of γ-globulin.[1,24] Thus a low λ_0 of 220 mμ obviously can be caused by either a certain folded or an unfolded conformation. In the instance of serum albumin, and most other cases (group I), however, the dispersion constant is a safer criterion than the specific rotation.[12]

On the basis of these facts, the following general conclusions can be made:

(1) The wide range of the dispersion constants of the native globular proteins (180–280 mμ) suggests a *variety of ordered compact conformations*. It is suggested that this variety depends, in the first place, on the *amino acid composition* and *primary structure* (residue sequence) of the proteins. While in simple polyamino acids a helix-coil model may sufficiently explain the variations in conformation, in proteins other conformations are indicated by the rotatory dispersion data. The secondary and tertiary structure (conformation) of proteins depends not only on the intra- and inter-chain hydrogen bonds but also on the amount and distribution of the hydrophobic amino acid residues, amount and distribution of electrically charged groups, and on the presence of covalent intra- and inter-chain bonds.[11,12,19,25]

(2) The convergence of the dispersion constants of the various proteins towards a common value of 210–220 mμ on disorganization of the native unique conformation suggests that the *chain conformation* is the dominant factor in determining the dispersion constant, and that a *random-chain conformation* is common to all globular proteins in disorganized state.

Note added in proof: Ch. Tanford, P. K. De and V. G. Taggart have recently published a paper [*J. Amer. Chem. Soc.* **82,** 6028 (1960)] on the rotatory dispersion of β-lactoglobulin in various solvents. These authors arrived at the conclusion that β-lactoglobulin, in spite of the fact that its dispersion constant in aqueous solutions is 240 mμ, must be considered as a non-helical protein. The α-helical conformation in the instance of this protein, according to Tanford *et al.*, is formed only in organic solvents. The absence of the α-helical conformation is explained as being due to the fact that the folding of the chains is caused not by intrachain hydrogen bonding but by the tendency of the lyophobic side chains to escape water. According to Tanford *et al.*, all of the proteins of class III and most of the class II are non-helical in aqueous solutions.

Summary—New and earlier published results on the optical rotatory dispersion of 42 globular proteins are tabulated in this paper. It was found that:

(1) The simple one-term Drude equation holds in all instances of native and denatured proteins. (None of the 42 proteins had absorption bands in the visible spectrum, and the specific rotation was measured in the wave length range of 334–600 mμ).

(2) The dispersion constants of native globular proteins vary within a wide range of 180–280 mμ. Since the dispersion constant is determined by the conformation of the polypeptide chains in the macromolecules of proteins, a variety of unique conformations is indicated in native globular proteins. A low dispersion constant is not necessarily associated with an unfolded and disorganized state.

[24] C. G. Schellman, Y. K. Prasad, and J. A. Schellman, *Abstr. Amer. Chem. Soc.* p. 44c, Natl. Meeting, San Francisco, April (1958).

[25] B. Jirgensons, *Natural Organic Macromolecules.* Pergamon Press, London, New York (1961).

(3) The dispersion constants of all denatured (disorganized) proteins approach a common value of 210–220 mμ. This finding is in agreement with the principle that the conformation of the chain determines the dispersion constant, and that denaturation leads to a disordered expanded chain conformation.

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23. B. Jirgensons, *Arch. Biochem. Biophys.* **74,** 57 (1958).
24. B. Jirgensons, *Arch. Biochem. Biophys.* **74,** 70 (1958).
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32. B. Jirgensons, T. Ikenaka and V. Gorguraki, *Makromol. Chem.* **39,** 149 (1960).
33. B. Jirgensons, *Arch. Biochem. Biophys.* **91,** 123 (1960).

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THE ROTATORY DISPERSION OF AMINO ACIDS

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ROTATORY dispersion studies on amino acids can reveal little concerning their structures since these are now so well established. In fact absolute configurations are now known for fairly complicated amino acids such as isoleucine, hydroxyproline, etc. It is only in relation to larger problems such as the polypeptides that such an investigation becomes of interest. I would like to review the line of thought which led to the group of experiments I am about to describe.

We are all aware of the very prominent work being done in the field of sequence determinations. It seems a great stroke of luck that, in many cases, optical rotation measurements ignore precisely this aspect of protein structure and respond instead to backbone configuration. This is only a working hypothesis but one gains confidence in its utility from the uniform rotatory properties of several synthetic polypeptides and the correlations established by Doty, Yang and Blout with the extensive measurements on proteins. A number of polypeptides known to be in helical form follow the same kind of dispersion curve, which is in accordance with the suggestion of Cohen that there is a distinct type of rotation attributable to the backbone helix *per se*.[1] Polypeptides and proteins known to be random follow another kind of curve. The properties of intermediate configuration can be deduced and are observed in many proteins.[2]

On the other hand optical rotation is a spectral property and one encounters many snags in this kind of interpretation. For example polybenzyl aspartate, polyhistidine, polytryptophane, etc. do not follow the rotatory dispersion that is believed established for right-handed helices. Bence–Jones proteins, pepsin and γ-globulin have a different kind of rotatory dispersion than the proteins first mentioned.† Finally with polypeptides and proteins containing large fractions of chromophoric or configurationally unusual side chains, specific residue effects can be expected.

Data on polypeptides and co-polypeptides are accumulating at a fast rate but it will certainly be some time before all polypeptides of interest will be investigated. We decided on the less reliable but very much faster approach of studying a large number of the amino acids themselves in order to detect as rapidly as possible the contribution of their side chains to the dispersion. The inference is not to be drawn that there will be a one to one correspondence between anomalous contributions in free amino acids with those in high molecular weight compounds, but a side chain chromophore which is highly active in an amino acid ought to be regarded as suspect in polypeptides until appropriate evidence proves otherwise. In the course of these studies a number of observations on the intrinsic rotatory capabilities of groups resulted.

* Chemistry Department, University of Minnesota, Minneapolis, Minn.
† See paper of B. Jirgensons in this volume, p. 166.

[1] C. Cohen, *Nature, Lond.* **175**, 1929 (1955).
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[3] C. Djerassi, *Optical Rotatory Dispersion*. McGraw-Hill, New York (1960).

Experimental conditions

The bulk of our observations were performed in acid solution. Actual conditions varied to suit the requirement that the carboxylate group was completely titrated to COOH so that the rotation was on a plateau with respect to pH and was little affected by small changes in pH or concentration. In this region the bulk of the rotation appears rather clearly to be associated with the COOH chromophore at 2050 Å* *except* when other chromophores of higher wave length are present. Simpson at this meeting has stated that this transition is the same as the 2900 Å transition of the CO group of ketones so much discussed in the papers of Djerassi and Klyne.

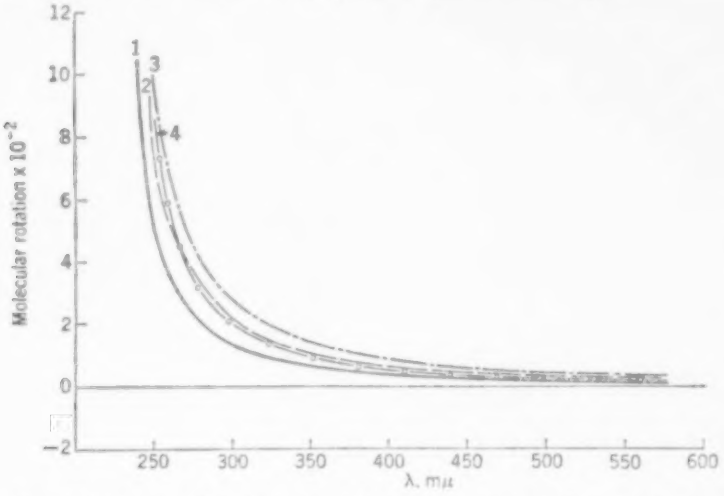

FIG. 1. RD curves of alkyl-substituted alanines: (1) alanine; (2) γ-aminobutyric acid; (3) valine, and norvaline; (4) leucine.

Results.

In order to make as brief as possible the task of presenting a large quantity of data, most of which is happily repetitious, the amino acids will be divided into groups. Readers who are unfamiliar with the structures of the amino acids will find them in a glossary at the end of this paper.

Group 1

Amino acids with no chromophores beyond the β carbon. We define a chromophore as a group with an absorption band above 1500 Å. Fig. 1 shows the type of data which was obtained. The curves are uniform and the substances involved are shown in the collective Table 1.

Group 2

Amino acids with chromophores beyond the β carbon atom. A typical set of data is shown in Fig. 2. Other examples differing little in dispersion will be found in Table 1. It would appear that the side-chain groups are sufficiently far from the COOH group in this case that they do not differentiate these substances from Group 1. Unfortunately no chromophores of the very near U.V. were studied. These would have provided

* This point is discussed in Ref. 3, Chapter 15.

TABLE 1. GROUPING OF AMINO ACIDS ACCORDING TO STRUCTURAL FEATURES IMPORTANT TO ROTATORY PROPERTIES

Group 1: aliphatically substituted amino acids	Group 2: amino acids lacking chromophore on β-carbon atom	Group 3: amino acids with chromophore on β-carbon atom	Group 4: amino acids with two asymmetric centers	Group 5	Group 6
Alanine	Methionine	Serine	Isoleucine	*Proline	*Cystine and derivatives
Aminobutyric acid	Arginine	Cysteine	*Threonine	*Hydroxy-proline	
Norvaline	Ornithine	Aspartic acid			
Valine	Lysine	Asparagine			
Norleucine	Glutamic acid	*Tyrosine			
Leucine		*Phenylalanine			
		*Tryptophan			
		†Histidine			
		Cysteic acid			
		*S-Ethylcysteine			
		*S-Methylcysteine			

* The dispersion curves for these substances are qualitatively as well as quantitatively distinct from alanine which shows a plain positive curve.
† Histidine shows anomalous behavior in alkaline, but not in acid solution.

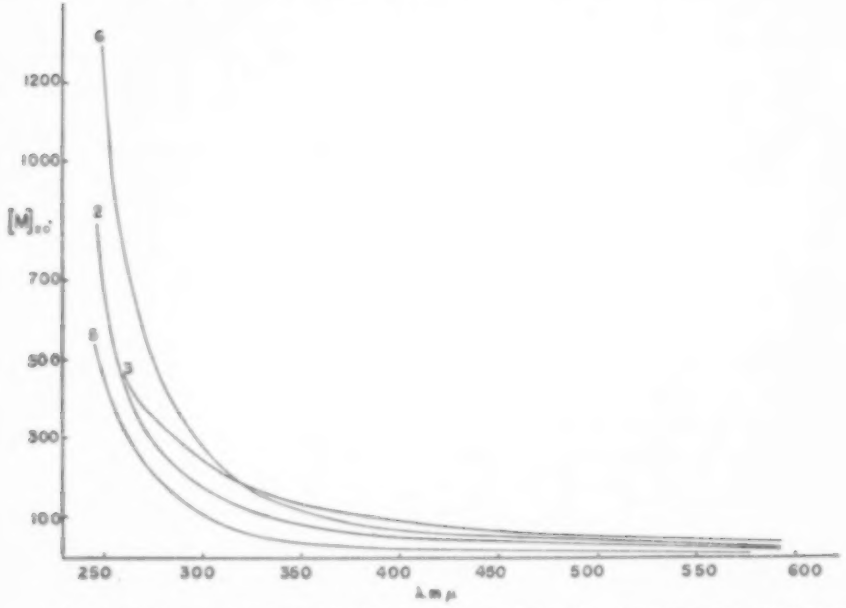

FIG. 2. RD curves of amino acids with chromophores beyond the β carbon atom; (1) alanine (reference); (2) arginine; (3) methionine; (4) lysine; (5) glutamic acid.

an additional stringent test of the effect of distance along the chain, though no common biological materials are of this kind.

Group 3

β-substituted chromophores. These are of two kinds: those which give rise to anomalous dispersion, and those which produce plain curves similar to groups 1 and 2. The latter are shown in Fig. 3. Individualism is beginning to appear but above 3500 Å

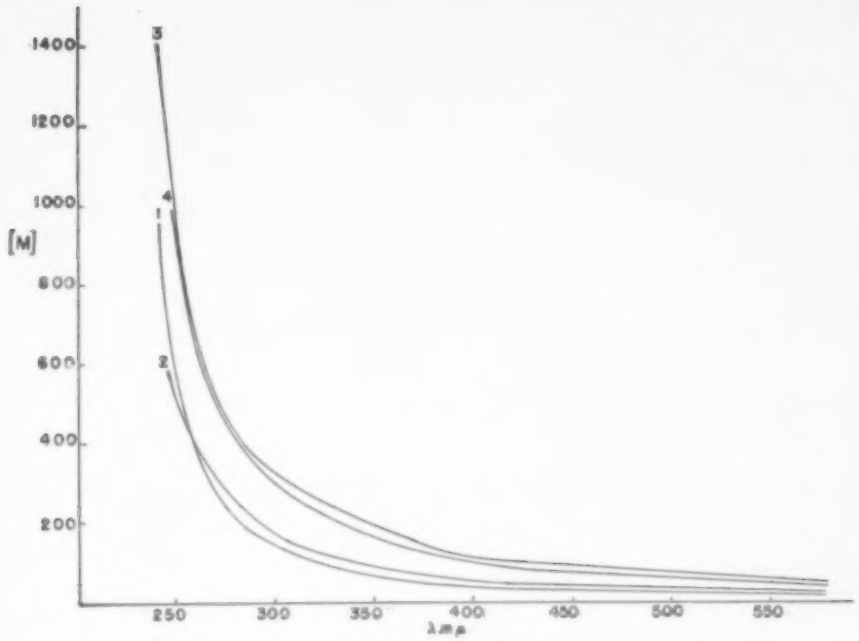

FIG. 3. RD curves of amino acids with β chromophores in the deep ultraviolet. (1) serine; (2) cysteine; (3) aspartic acid; (4) cysteic acid.

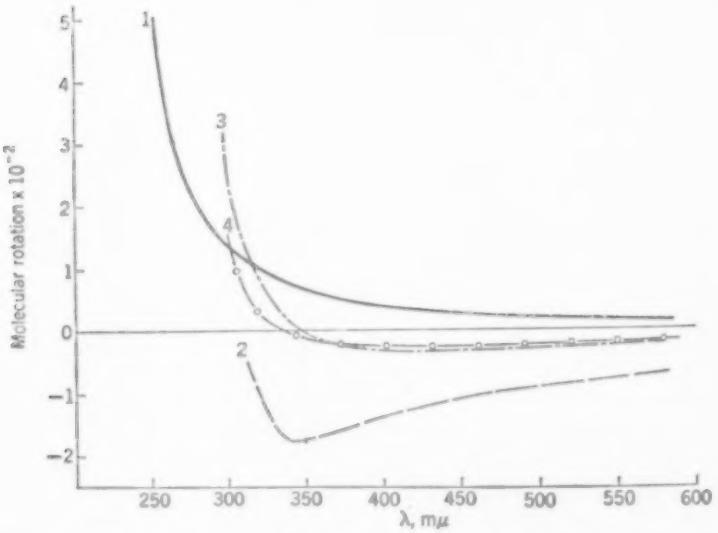

FIG. 4. RD curves of amino acids with β chromophores in the near ultraviolet. (1) alanine (reference); (2) tryptophane; (3) tyrosine; (4) phenylalanine.

where most polypeptide and protein dispersion runs are performed, the curves are definitely banded together. On the other hand phenylalanine, tyrosine and tryptophane show distinctly anomalous behavior (Fig. 4), very likely arising from their absorption bands above 2500 Å. Cotton effect curves could not be obtained because of absorption but two term dispersion curves calculated with the formulas of Lowry[4] led in each case to a λ_0 corresponding to the appropriate absorption band. For tyrosine similar results had been obtained over a more limited wave length region. Ethyl and methyl cysteine also gave anomalous curves at 20°. Both are not reproduced, but Fig. 5 displays an unusual feature of the temperature dependence of the dispersion of S-ethyl cystine: the curve is anomalous at 20° with a broad minimum at about 4750 Å, and simple at 50°. It seems reasonably plain from the closeness of the curves that this does not represent a structural change but a gentle raising of the curve across the zero axis. Results of this kind point out a warning against attributing special significance to anomalous dispersion.

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Group 4

Amino acids with more than one asymmetric center. Unusual features might be expected here and they are observed. The threonine dispersion curve is displaced downward from the norm to become anomalous; the isoleucine curve is displaced upwards to be unusually dextrotatory.

Group 5

Proline and hydroxyproline. Here again the curves deviate from the norm (Fig. 7). The amino acids are levorotatory, the trough of a Cotton effect curve is observed, and the rotations are very large, in accordance with the rule of Kauzmann and Eyring[5] for ring structures. Pyroglutamic acid also shows these features (unpublished results).

Group 6

Cystine. Cystine has long been known as one of the many disulfide compounds which show exceptionally large rotations at the sodium D line. Fig. 8, which compares the dispersion of cystine and alanine demonstrates not only the great magnitude of the rotation at all wave lengths, but shows the trough of a Cotton wave of very large amplitude at 2650 Å. This is undoubtedly associated with the absorption band of cystine at 2500 Å*, and demonstrates that the bulk of the optical rotation is associated with the disulfide group itself. This probably arises from the fact that the disulfide link is intrinsically asymmetric as is shown in Fig. 9. The 90° dihedral angle gives rise to a screw sense in all disulfides. With no asymmetric center, right and left senses occur with equal probability. But since both R groups in cystine are asymmetric, it is more than conceivable that one or the other configuration will be preferred, thus producing an intrinsic rotation associated with the disulfide link. It is interesting that the Cotton effect is positive in homocystine (unpublished observations).

* Some disulfides have absorption maxima and Cotton curves at higher wave lengths. See references to Schotte and Sjoberg.

[4] T. M. Lowry, *Optical Rotatory Power*. Longmans, Green, London (1935).
[5] W. Kauzmann and H. Eyring, *J. Chem. Phys.* 9, 41 (1941).

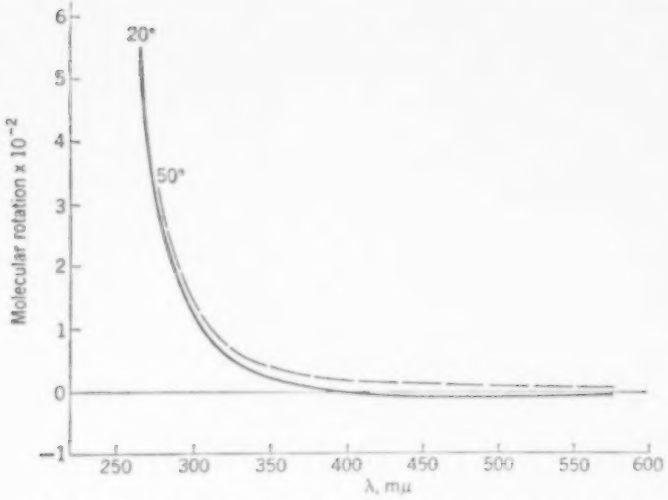


FIG. 5. Effect of temperature on the rotatory dispersion of S-ethylcysteine.

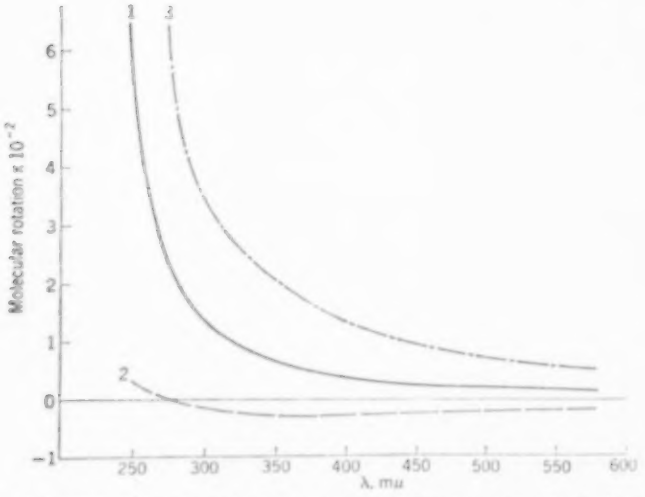


FIG. 6. RD curves of amino acids with two centers of asymmetry: (1) alanine (reference); (2) threonine; (3) isoleucine.

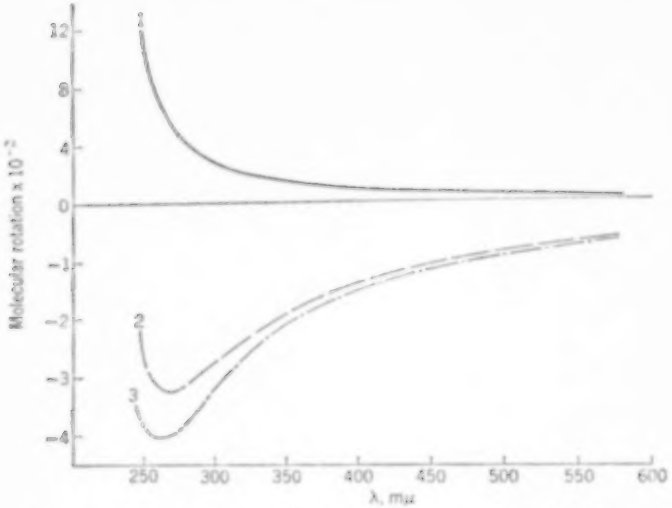


FIG. 7. RD curves of (1) norvaline (reference); (2) proline; (3) hydroxyproline.

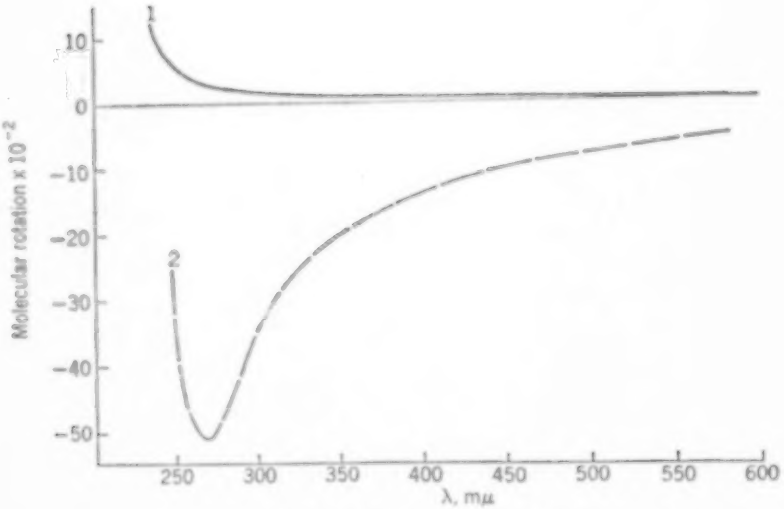


FIG. 8. RD curves of (1) alanine; (2) cystine.

Discussion

First, it must be emphasized that a comparison between the side chain contribution of a chromophore in an amino acid and in a polypeptide is not a rigorous procedure. In an amino acid the chromophore "sees" a very different set of vicinal groups than in a polypeptide, especially if the polypeptide is in the helical conformation.

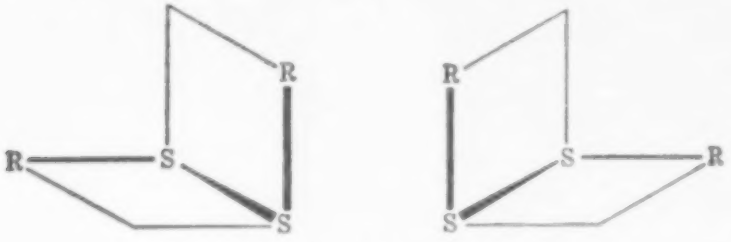


FIG. 9. The asymmetric disulfide group.

Consequently these studies should be regarded more as a scouting expedition than the firm establishment of fact. Yet they have their value because the only way in which the contribution of a chromophore to the dispersion can be established or eliminated in high molecular weight compounds is by the measurement of dispersion within its absorption band and this is difficult work. To my knowledge only two investigations of this kind have been carried out, one on proteins,[6] and the other on polypeptides reported in Blout's paper at this meeting*.

The overall results are shown in Table 1 which lists most of the amino acids which have been investigated. Substances marked with asterisks are those whose dispersion curves are qualitatively as well as quantitatively distinct from alanine which shows a plain positive curve. (Glycine, of course, also falls into the category of an amino acid with unusual rotatory properties.)

It appears to us that it is rather a dangerous procedure to correlate rotatory dispersion directly with backbone conformation with any polypeptide or protein containing relatively large quantities of these amino acids.

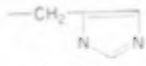
Roughly 25 per cent of the natural amino acids are of the "anomalous" variety. Yet an inspection of the amino acid composition of most proteins reveals that they usually contain at least 90 per cent of the alanine-like amino acids. Thus the above measurements are in accordance with a conformational interpretation of rotatory dispersion. One investigation of dispersion on serum albumin and ribonuclease between 2500 and 3000 Å[6] revealed that the contribution of the aromatic side chains is negligibly small compared to the bulk rotation of the backbone, presumably because of their small mole ratios in these proteins. In contrast Würtz and Haurowitz[7] have been recently investigating the effect of disulfides on total rotation in protein and have concluded that the contribution is significant.

* *Note added in proof:* Recently Simmons, Cohen, Szent-Gyorgyi, Wetlaufer and Blout have extended their measurements below 2500 Å and have observed Cotton effects originating from the peptide bond with a number of proteins and helical polypeptides. *J. Amer. Chem. Soc.* In press.[7]

[6] J. and C. Schellman, *Arch. Biochem. Biophys.* **65**, 58 (1956).
[7] H. Würtz and F. Haurowitz, *Abstract of Paper Presented at the American Chemical Society Meeting*, Cleveland (1960).

One final instance of where conformational rules might break down is in the presence of a prosthetic group which absorbs in the visible; e.g. heme groups and colored metal complexes. We have seen in Eichorn's paper that their bands are optically active and their proximity to the region of measurement could well cause them to dominate, completely obscuring the conformational aspects of dispersion.

GLOSSARY OF STRUCTURES OF AMINO ACIDS

R	Amino acid
$-CH_3$	Alanine
$-CH_2CH_3$	Aminobutyric acid
$-(CH_2)_2CH_3$	Norvaline
$-(CH_2)_3CH_3$	Norleucine
$-CH(CH_3)_2$	Valine
$-CH_2CH(CH_3)_2$	Leucine
$-CH(CH_3)CH_2CH_3$	Isoleucine
$-CH_2OH$	Serine
$-CH_2SH$	Cysteine
$-CH_2CO_2H$	Aspartic acid
$-CH_2CONH_2$	Asparagine
$-CH(OH)CH_3$	Threonine
	Histidine
$-CH_2C_6H_5$	Phenylalanine
$-CH_2C_6H_4OH$	Tyrosine
	Tryptophan
$-CH_2SO_3H$	Cysteic acid
$-CH_2SCH_3$	S-Methylcysteine
$-CH_2SC_2H_5$	S-Ethylcysteine
$-CH_2CH_2CO_2H$	Glutamic acid
$-CH_2CH_2SCH_3$	Methionine
$-(CH_2)_3NHC(=NH)NH_2$	Arginine
$-(CH_2)_3NH_2$	Ornithine
$-(CH_2)_4NH_2$	Lysine
$-CH(CO_2H)CH_2CO_2H$	Amino tricarballylic acid

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OPTICAL ROTATORY PROPERTIES OF POLYNUCLEOTIDES AND NUCLEIC ACIDS

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THE nucleic acids are currently a major focal point of investigation for the molecular biologist. This is because it is now recognized that this group of macromolecules plays a central role in the hereditary process, and in the dissemination of the genetic information within a given generation.

In a search for better ways to reveal some of the more subtle aspects of nucleic acid conformation in solution, a number of laboratories[1-5] have recently turned to the method of optical rotation. It is not difficult to understand this, when one considers the success with which this tool has been employed within the last decade to detect and quantitate α-helical secondary structure in synthetic polypeptides and proteins.[6-8] Indeed, from this work it has become abundantly apparent that optical rotation can reflect regularities in main chain configuration which are not directly related to asymmetric atoms. Now, in the case of nucleic acids, there exist similar possibilities for optical rotatory effects arising from secondary structure; for their covalent nature would seem to require that structural regularity will arise from some form of helical distribution of the polar groups in the backbone, due to the need to maintain maximum charge separation.

It will become apparent from what follows that our understanding of the optical rotatory properties of nucleic acids has not reached the degree of refinement achieved in the case of polypeptides. Indeed, there are wide gaps even at the observational level. Nevertheless, a number of generalities are beginning to emerge, and it is to these positive aspects that I direct my attention.

Centers of optical activity in nucleic acids

Primary structure. A priori, one might expect nucleic acids to show a baseline level of optical activity, merely due to the presence of asymmetric atoms. These will include several carbon atoms in the pentose of each nucleotide (monomer unit), but not the phosphorus atom involved in the internucleotide linkage, since the latter contains two equivalent oxygens. The disposition of atoms in a polynucleotide chain may be seen in Fig. 1, which shows a section of a hypothetical ribonucleic acid (RNA) chain. The backbone of deoxyribonucleic acid (DNA) differs from that shown only in that the pentose C-2, in lacking oxygen, is no longer asymmetric.

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[1] P. Doty, H. Boedtker, J. R. Fresco, R. Haselkorn and M. Litt, *Proc. Natl. Acad. Sci.* **45**, 482 (1959).
[2] J. R. Fresco, *Trans. N.Y. Acad. Sci.* Ser II, **21**, 653 (1959).
[3] B. H. Levedahl and T. W. James, *Biochem. Biophys. Acta* **17**, 453 (1955).
[4] A. Pour-El and C. A. Dekker, *Abstr. Amer. Chem. Soc. 136th Meeting* p. 7c (1959).
[5] P. Ts'o, *Tetrahedron.* This volume(1961).
[6] P. Doty, *Proceedings of the Fourth International Congress of Biochemistry* Vol. IX, p. 8. Pergamon Press, London (1959).
[7] E. Blout, *Tetrahedron.* **13**, 123 (1961).
[8] J. T. Yang, *Tetrahedron.* **13**, 143 (1961).

The fully randomly coiled polynucleotide chain is, nevertheless, not to be expected to display optical activity predictable simply from that of its pentose constituent. For it has been observed that the base substituent on the pentose while not optically active by itself has a significant effect on the resulting nucleoside.[9,10] Thus, whereas those with purine substituents have $[\alpha]_D$ values somewhat negative, pyrimidine nucleosides

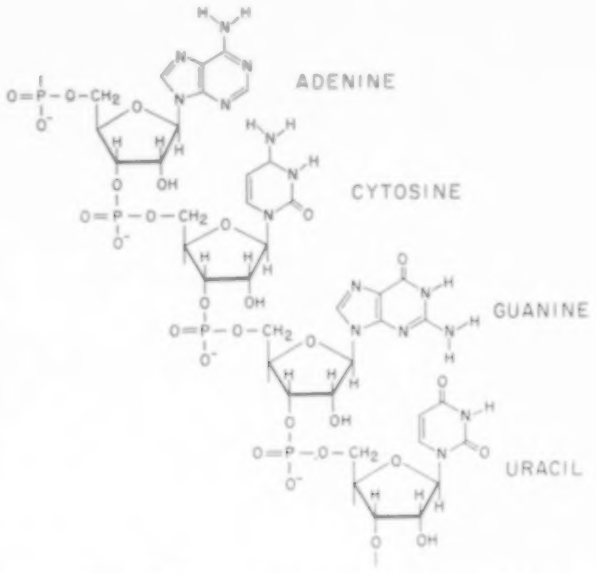

FIG. 1. The chemical structure of ribonucleic acid.

show small positive rotations. In general, also, those nucleosides containing D-2-deoxyribose have an $[\alpha]_D$ about 20° more positive than their D-ribose counterparts.[11] The introduction of a phosphate group to the 3′ or 5′ position of a nucleoside has a small and apparently unsystematic effect on the rotatory power.* From all this, we can predict that for a completely disordered polynucleotide chain, be it of the RNA or DNA type, the $[\alpha]_D$ will be in the region of 0°.

Secondary structure. What, then, are we to expect when the polynucleotide back-bone is brought into some type of ordered helical conformation? A number of multi-stranded *completely* helical polynucleotides are now known. These include DNA and several synthetic polyribonucleotide complexes†, e.g. poly(A + A)[12–14]

* Unfortunately, the data on nucleosides and nucleotides are sparse and sometimes contradictory. These generalities have been made after careful selection of what seemed to the author to be the most reliable data.

† The following abbreviations have been used. A = adenine; G = guanine; U = uracil; C = cytosine; I = inosine, where the base is hypoxanthine. The prefix *poly* is used to indicate a homo or copolymer of nucleotide(s) of the indicated base(s). Polynucleotide complexes are indicated by the prefix *poly* followed in parentheses by the summated homopolymer chain symbols.

[9] P. A. Levene and E. S. London, *J. Biol. Chem.* **83**, 793 (1929).
[10] B. H. Levedahl and T. W. James, *Biochim. Biophys. Acta* **26**, 89 (1957).
[11] J. Baddiley in *The Nucleic Acids* (Edited by Chargaff and Davidson) Vol. I, pp. 156, 178. Academic Press, New York (1955).
[12] J. R. Fresco and P. Doty, *J. Amer. Chem. Soc.* **79**, 3928 (1957).
[13] J. R. Fresco and E. Klemperer, *Ann. N.Y. Acad. Sci.* **81**, 739 (1959).
[14] R. Steiner and R. Beers, *Biochem. Biophys. Acta* **32**, 166 (1959).

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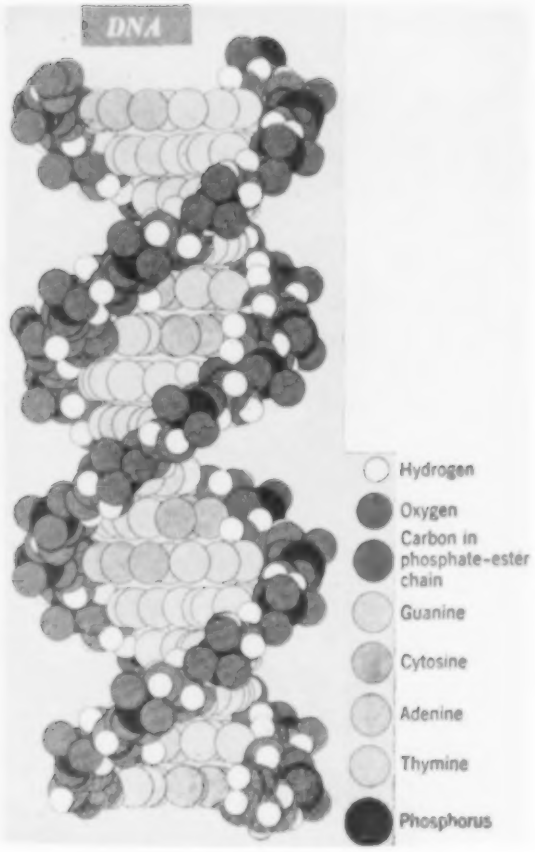

FIG. 2. A schematic representation of the two-stranded deoxyribonucleic acid helix.[204]

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poly(A + U),[15–17] poly(A + U + U),[17] poly(I + C),[18] poly(I + I + I).[19] Of these, the most detailed structure analysis is available for DNA,[20,21] whose comformation is schematically presented in Fig. 2. In this complex, as in all the others, the pentose-phosphate backbone is on the periphery of the helix, and the base residues are hydrogen-bonded in pairs or triplets in the interior. In all these cases, then, additional contributions to the optical rotation may appear due to the helical conformation. One of these should arise from the interaction of the polarized light with the optically active centers in the helically wound backbone strands. It is useful, perhaps, to think of the pentose-phosphate backbones as analogous to the α-helix backbone of the polypeptide, recognizing, however, that the polynucleotide rotation arises from D residues, whereas the polypeptide contains L residues. In the polynucleotide, we might expect, another conformational effect to result from the helical array of the base pairs, which can be thought of as a series of discs translating about 36° every 3·4 Å (or some similar repeat distance in the other complexes) along the helix axis. The contribution due to these chromophores could, in fact, be quite large, if as might be supposed, the base pairs involve one or more $n \rightarrow \pi^*$ transitions[22,23] in addition to $\pi \rightarrow \pi^*$ transitions.†

Dependence of specific rotation on conformation

The helix-coil transition. A large body of physico-chemical evidence is now available which shows that the increases in ultraviolet absorption at 260 mμ that accompany the exposure of helical polynucleotides in solution to elevated temperatures provide a direct measure of the denaturation process.[1] Thus, a plot of ultraviolet absorption versus temperature reflects the *melting* of the helical conformation into a disordered random coil.

This ultraviolet absorption change is undoubtedly related to the fact that in organized polynucleotides, the absorption of the constituent nucleotide units is markedly depressed.[13,15,17,24] This hypochromicity appears to be due to the particular mode of stacking of the bases maintained as a result of hydrogen bond interactions. Thus, when thermal disruption of the hydrogen bonds occurs, the absorbing properties of the chromophores revert almost completely to those of the individual monomer units.[25]

In view of this, and in an attempt to assess the contribution of helical conformation to rotatory power, we investigated the changes in specific rotation which could be thermally induced in several helical polynucleotides.[1,2,13] The results for DNA and poly (A + U) are shown in Figs. 3 and 4. For the sake of comparison, the optical

† It is observed generally that $n \rightarrow \pi^*$ transitions contribute more strongly to rotatory power than do $\pi \rightarrow \pi^*$ transitions, although their inherent absorbancy is at least an order of magnitude lower.

[15] R. C. Warner, *J. Biol. Chem.* 229, 711 (1957).
[16] A. Rich and D. R. Davies, *J. Amer. Chem. Soc.* **78**, 3548 (1956).
[17] G. Felsenfeld and A. Rich, *Biochim. Biophys. Acta* **26**, 457 (1957).
[18] D. R. Davies and A. Rich, *J. Amer. Chem. Soc.* **80**, 1003 (1958).
[19] A. Rich, *Biochim. Biophys. Acta* **29**, 502 (1958).
[20a] M. Feughelman, R. Langridge, W. E. Seeds, A. R. Stokes, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins, R. K. Barclay and L. D. Hamilton, *Nature, Lond.* **175**, 834 (1955).
[20] F. H. C. Crick and J. D. Watson, *Proc. Roy. Soc.* A **233**, 80 (1954).
[21] R. Langridge, W. E. Seeds, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins and L. D. Hamilton, *J. Biochem. Biophys. Cyt.* **3**, 767 (1957).
[22] H. Murakami, *J. Chem. Phys.* **27**, 1231 (1957).
[23] M. Kasha in *Light and Life* (Edited by W. D. McElroy and B. Glass). Johns Hopkins, Baltimore (1960).
[24] A. Thomas, *Biochim. Biophys. Acta* **14**, 231 (1954).
[25] J. R. Fresco, R. Haselkorn and P. Doty, unpublished.

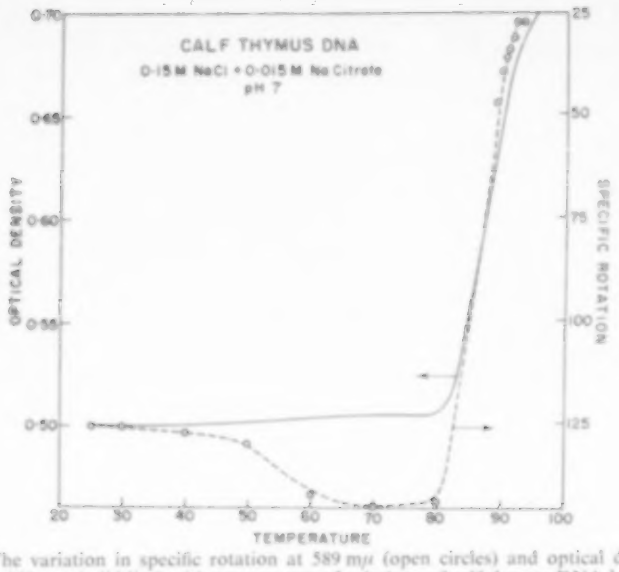

FIG. 3. The variation in specific rotation at 589 mμ (open circles) and optical density at 259 mμ (solid line) with temperature of solutions of calf thymus DNA.[1]

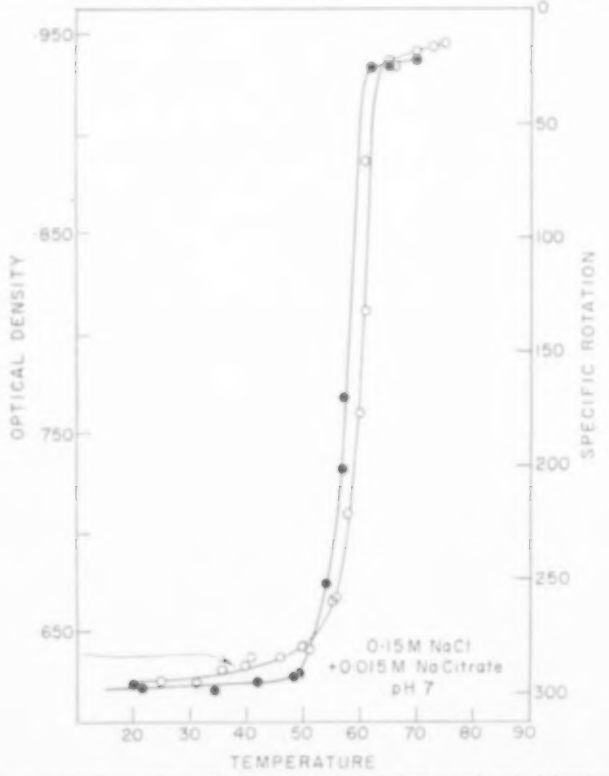

FIG. 4. The variation in specific rotation at 589 mμ (filled circles) and optical density at 260 mμ (open circles) with temperature of solutions of poly (A + U).[1]

density data are also plotted and the two ordinates are normalized. It should be noted that since there are reversible aspects to the transitions, all measurements were made at the ambient temperature. From such data, of which the examples shown are typical, it is clear that optical rotation does depend on conformation.

At a glance, it is evident that the profiles obtained by the two optical methods are essentially parallel. Also, at high temperature, where the polynucleotide chains are disordered coils, the $[\alpha]_D$ approaches 0°, which is consistent with our earlier argument. Finally, it can be seen that the contribution due to helical conformation is a very sizable *positive* one. Since the sign appears to be the same in all the polynucleotides so far

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TABLE 1. OPTICAL ROTATION OF POLYRIBONUCLEOTIDES

Polymer	Temperature	$[\alpha]_D$
Poly U—single strand	22°	−8°
Poly A—single strand	22°	155°
	90°	−5°
+ 8M urea	22°	75°
Poly (A + A)—helix	22°	300°
Poly (A + U)—helix	22°	300°
	70°	23°

The solvent is neutral saline solution of 0·2 ionic strength in all cases except for poly (A + A), where the pH is 4·9.

studied, it seems reasonable to conclude that all the helices, like DNA, whose screw sense has been independently established,[20] must be righthanded.

The DNA profile contains two characteristic features which set it apart from those of the polyribonucleotides. One concerns the dip, or increase in positive rotation, in the temperature region between 30 and 80°. It must be emphasized that this is a reproducible observation, and it has also been noted when a similar profile was determined at 289 mμ, in the region of the Cotton effect (see below). This finding suggests that as the temperature is raised, there are subtle changes in the geometry of the molecule, perhaps in the pentose constituent, to which ultraviolet absorption is insensitive. The second point is the much lower magnitude of the positive rotation in native DNA. A simple explanation for this is also not immediately apparent, although it seems possible that 589 mμ, the D line, may represent a position more distant from the critical wave length in the deoxyribose than in the ribose polynucleotides.

Another way to emphasize the effect of helical structure on rotatory power is to compare the specific rotation of a multistranded helix with that of its single-stranded polyribonucleotide components. Some relevant data are presented in Table 1. Consider first the poly (A + A) helix. At room temperature, where this complex is stable, it has an $[\alpha]_D$ of 300°, which is about 145° higher than that of its single-chain component. The poly (A + U) helix presents an even more striking picture. In this instance, the interaction of one strand having an $[\alpha]_D$ of −8° (poly U) with another having an $[\alpha]_D$ of 155° (poly A) results in a helical complex with an $[\alpha]_D$ of 300°. Apparently, when the poly U strand assumes a helical conformation, it contributes with as high a positive rotation as the poly A chain.

Helical content of single-stranded polynucleotides. An obvious extension of the

finding that optical rotatory power reflects helical conformation in multistranded polynucleotide complexes was to exploit this property as a probe for helical order in single-stranded polynucleotides (including RNA).

Prior to this effort it was recognized from an assessment of their ultraviolet hypochromicity that there exists among these single chain polymers a gradation in degree of intramolecular hydrogen bonding. It now became evident that a similar gradation can be made on the basis of degree of positive rotation. Fortunately poly U at room

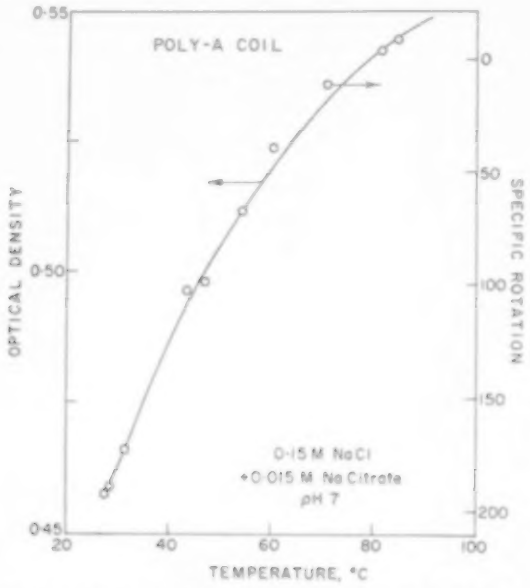

FIG. 5. The variation in specific rotation at 589 mμ (open circles) and optical density at 257 mμ (solid line) with temperature of neutral solutions of poly A single strands.[10]

temperatue provides baseline levels for both these parameters, i.e. it shows almost no ultraviolet hypochromicity, and consistent with this, it has an $[\alpha]_D$ very close to that of its monomer. Poly A, on the other hand, displays considerable hypochromicity, and as shown above, has an $[\alpha]_D$ intermediate between that of the two-stranded helical form, and that of the monomer (whose value is approached when the temperature is raised near 100°). When 8 M urea is added to the solvent, so that a large proportion of the intramolecular hydrogen bonds in poly A are broken at room temperature, there is a marked decrease in the hypochromicity and a concomitant decrease in positive rotation. Indeed, the course of the changes in hypochromicity which can be induced over the accessible temperature range is completely paralleled by decreases in specific rotation (see Fig. 5). Similar observations have been recorded for RNA from different sources (Fig. 6) and for single-stranded denatured DNA (Fig. 7).

From such observations, we are prone to conclude that nearly all the hydrogen bonded bases of single-stranded polynucleotides are contained within helical regions. Naturally, this structural assignment does not rest merely on the qualitative coincidence of the rotatory and absorbance phenomena described above. Equally significant and more revealing are the magnitudes of the rotational effects. For these represent a

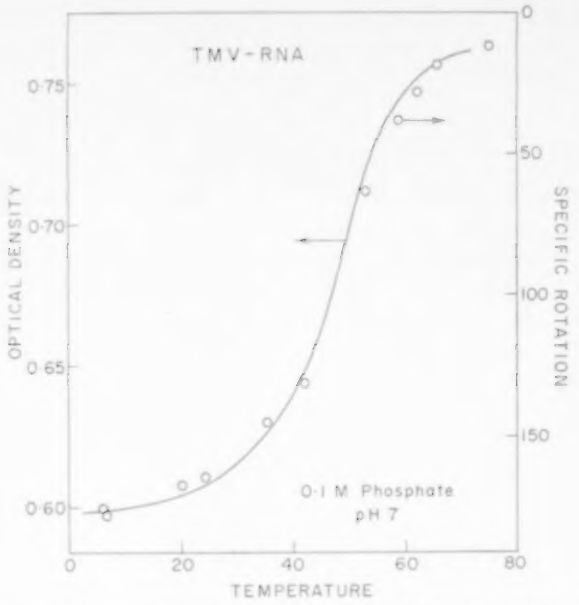

FIG. 6. The variation in specific rotation at 589 mμ (open circles) and optical density at 260 mμ (solid line) with temperature of solutions of Tobacco Mosaic Virus RNA.[3]

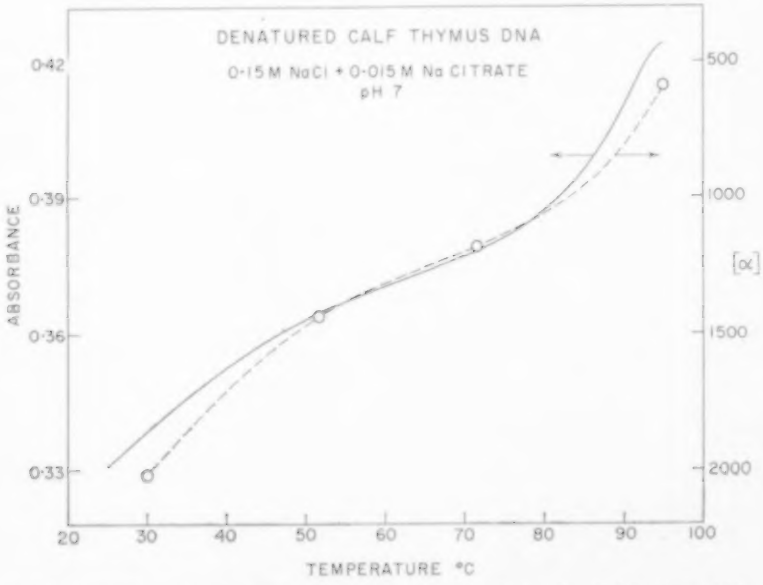

FIG. 7. The variation in specific rotation at 289 mμ (open circles) and optical density at 259 mμ (solid line) with temperature of solutions of heat denatured DNA.[2a]

substantial proportion of the changes elicited when a multistranded complete helix undergoes transition to the random coil. In fact, they closely approximate the values predicted from the product of the fraction of hydrogen-bonded bases (adduced from hypochromicity data) and the rotational change expected from the melting of a completely helical polynucleotide.[1]

It must be admitted, nevertheless, that the estimation of helical content of single-stranded polyribonucleotides from specific rotation data depends in some measure on three assumptions: (1) that the nature of the helical regions within the single strand is much like that of the complete helical complexes; (2) that the length of a helical region does not markedly affect rotatory power; and (3) that the types of hydrogen bonded base pairs in the helix are of little importance. There is now being amassed convincing evidence, not appropriately discussed here, that the first assumption is justified at least for RNA and synthetic polyribonucleotide co-polymers.[26] Unfortunately, we can only speculate on the second assumption, since our examination of this matter, using homogeneous oligonucleotides, has just begun. In any helix, an end effect may be expected which will result in a lowering of the average residue rotation. Obviously the significance of this factor diminishes rapidly as chain length increases, so that by the time a ten residue-pair helix is reached the effect is probably quite small. Since the stability–temperature curves of RNA indicate most helical regions are this length or greater,[26] it can be argued that this factor will not markedly distort the calculation of helical content. The validity of the third premise rests on the close similarity of the specific rotational changes which accompany the helix-coil transitions of poly(A + U), poly(A + A) and poly(U + U),[27], i.e. 275, 300 and 290° respectively at 589 mμ. Of course, since the helical regions in RNA probably involve A-U and G-C base pairs, our estimates will have to be considered tentative until data for a poly(G + C) helix becomes available. Nonetheless, it would seem from the examples cited that the scale being used will not be found to be off by more than 10 per cent. With these reservations then, and recognizing that most RNAs in neutral solution of moderate ionic strength display $[\alpha]_D$ values at room temperature in the range of 140—180°, we estimate that these values correspond to helical contents in the neighborhood of 40–60 percent.

Rotatory dispersion and the Cotton effect

The information presented thus far, derived as it is from a single measured parameter, specific rotation at a particular wave length (generally 589 mμ), provides a useful but nonetheless only an empirical elucidation of the dependence of rotatory power of polynucleotides on helical conformation. An extension of such measurements to a broad wave length range in order to delineate the character of the rotatory dispersion naturally suggests itself. Possibly, such data would be amenable to theoretical treatment, from which more refined molecular definition of nucleic acid structure could be achieved. In a more limited sense, rotatory dispersion might be expected to provide a sensitive way of amplifying the conformationally dependent effects revealed by the specific rotation data. For example, it might be found as has been observed in the case of polypeptides,[6–8], that the helical polynucleotides exhibit marked departures from the one-term Drude equation expected for the completely disordered forms. In this event, a more satisfactory means of quantitating helical content of single-stranded

[26] J. R. Fresco, B. M. Alberts and P. Doty, *Nature, Lond.* **188**, 98 (1960).
[27] M. Lipsett, *Proc. Natl. Acad. Sci.* **46**, 445 (1960).

polynucleotides could be anticipated. In connection with this, anomalous dispersion particularly associated with the region of absorption of the purine and pyrimidine bases might be expected, since, as was pointed out earlier, these chromophores are an integral part of the helical structure in nucleic acids. The study of such an anomaly, which would take the form of a Cotton effect, could lead to an exposition of molecular and electronic interactions related to base pairing.

In actual fact the realization of the potentialities inherent in rotatory dispersion data of nucleic acids is only now beginning to be effected. It may nevertheless be profitable to attempt some evaluation of the little amount of information at hand.

Nucleosides. The rotatory dispersion of the individual nucleoside components of DNA has been investigated[10] over the region 320–600 mμ, and found to conform in each case to the Drude equation. It is instructive to compare the λ_c values (λ_c is supposed to indicate the wave length of an optically active transition) reported in that study with the wave lengths of maximum absorption, λ_{max}, of the nucleosides, since in these relatively simple substances one might hope to sort out direct relationships between these two optical properties. The λ_c of adenine, cytosine and thymine deoxynucleosides coincide with their λ_{max}, suggesting that these two parameters are the result of a common transition. Consistent with this conclusion, the long wave length absorption bands of these nucleosides, 230–300 mμ, appear to be simple. At first sight the guanine nucleoside presents a somewhat different picture, for it has a λ_c about 20 mμ higher than its λ_{max}. This dilemma is readily interpreted, however, upon consideration of the guanosine absorption spectrum. This spectrum is not simple, having a distinct peak (at pH 7) at 253 mμ, and a prominent shoulder at longer wave lengths. The shoulder is probably due to a second band, about half the intensity of the one at 253 mμ, that has a λ_{max} corresponding very closely to the λ_c observed for guanosine. This shows that these two absorption bands differ very substantially in their optical activity.

It is evident from these observations that over the wave length range examined, the rotatory properties of nucleosides are dominated by the base linked to the pentose even though the base itself is not optically active. In view of this, it can be surmised that nucleosides will be found to display Cotton effects in the region 230–300 mμ.

Polyriboadenylic acid. Thus far, our examination of poly A has been limited to the region 360–600 mμ. Within this region all the data, regardless of the conformation of the polynucleotide, fit the one-term Drude equation. Nonetheless, the λ_c does vary with the degree of helical content in a regular manner as is evident from the following: in acid solution, where the completely helical form, poly(A + A) exists, $\lambda_c = 290$ mμ; in neutral salt solution of 0·2 ionic strength, where it is estimated that 50 per cent of the residues in the single chain are organized into short helical regions, $\lambda_c = 272$ mμ; and when urea is added to such a neutral solution of poly A so that about half of the helical regions are disrupted, the λ_c drops to 257 mμ, which is close to the λ_c of the monomer.

Apparently, helical secondary structure in poly A causes certain electronic transitions located on the long wave length side of the 230–300 mμ absorption band to be accentuated. These transitions are probably of the $n \rightarrow \pi^*$ type since they are in the weakly absorbing part of the spectrum, yet are highly optically active. In this connection we have observed that the absorption spectrum of poly A (when traced with a continuous recording spectrophotometer) contains some diffuse fine structure on the

long wave length side in the form of shoulders and slightly resolved peaks. This fine structure is proportional to the helical content, disappears when the helix is completely melted out, and is absent in the monomer.

Ribonucleic acid. The rotatory dispersion of RNA has also been shown to follow the Drude equation regardless of the helical content.[30] In this case, however, the λ_c changes from 250 mμ to 310 mμ upon going from the partially helical conformation to the completely random one. The direction of this change is opposite that which has been observed for poly A, and for the moment there is no ready explantion for this.

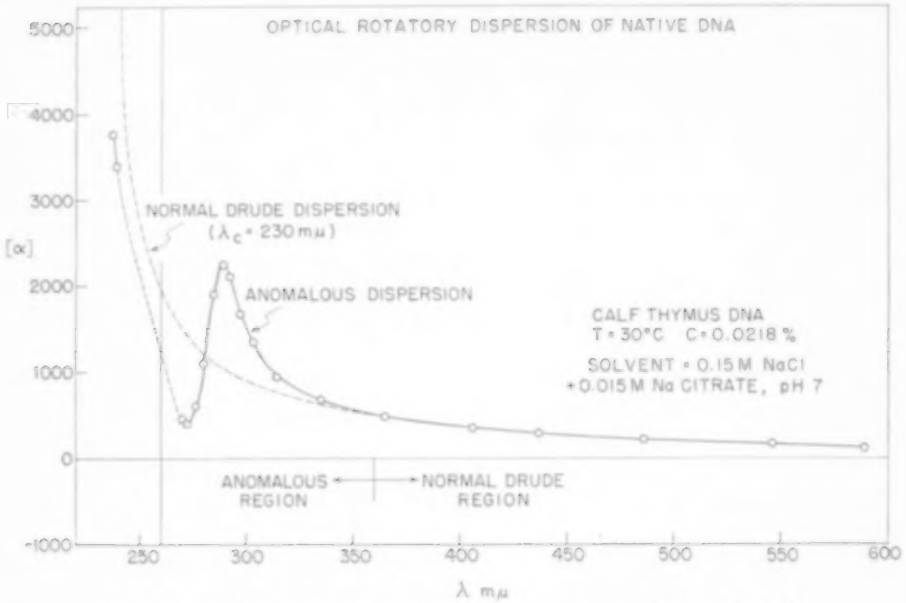

FIG. 8. The variation of specific rotation with wave length of calf thymus DNA.[28]

We can only speculate that in RNA the observed λ_c is a composite of a number of different optically active transitions resulting from a multiplicity of base pair sequences. It can be hoped that these will be resolved when it becomes possible to observe the rotatory dispersion within the region 230–300 mμ.

Deoxyribonucleic acid. Previous to our own recent efforts[2,28] it was reported[3] that the rotatory dispersion of DNA is of the simple Drude type between 320 and 700 mμ. The scatter in the λ_c values and the ill defined nature of the samples used in this work prompted us to reexamine the problem. Our investigations have taken two related directions. One aim was to delineate accurately the rotatory dispersion of native DNA in the readily accessible region above 320 mμ, and having done so, to examine the changes in the dispersion which accompany denaturation. Related to this, we wished to probe as far into the 230–300 mμ region as would be possible with the most intense light sources available in an effort to observe any Cotton effects which might occur there. Some progress can now be reported in both these directions.

A composite plot of our observations is given in Fig. 8. It can be seen that the

[28] J. R. Fresco and R. Gorn, unpublished.

dispersion is of the simple Drude type at longer wave lengths, but becomes anomalous below 360 mμ. Evidently, this anomaly is related to the prominent Cotton effect which becomes well defined below 300 mμ. To more sensitively demonstrate the region of Drude-type dispersion and ascertain the λ_c associated with it, the data are shown plotted in Fig. 9 according to the Yang modification[29] of the Drude plot. From the square-root of the slope of the straight-line portion, a λ_c of 230 was obtained, which is somewhat lower than the value quoted above for RNA.

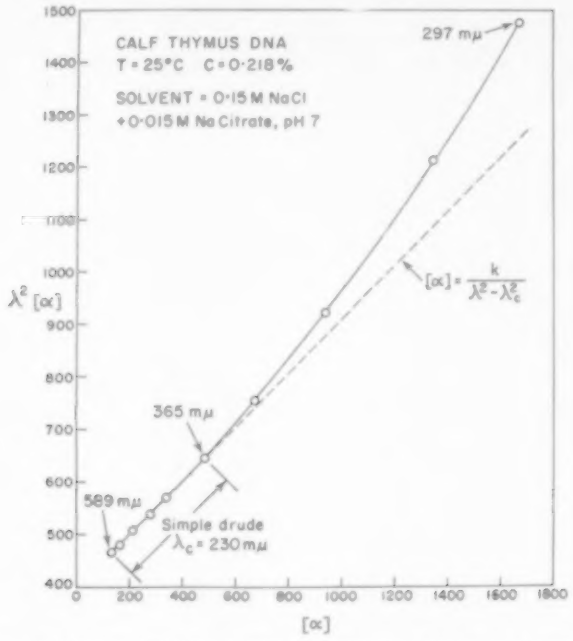

FIG. 9. A Yang-modified Drude plot of the rotatory dispersion of native DNA.[28]

Unfortunately, the measurement of optical rotation of nucleic acids in the region below 300 mμ is attended by considerable difficulty. A very intense light source is required for such work because of the high absorbance of the purine and pyrimidine residues. The measurements on DNA reported for wave lengths below 300 mμ were made using a quartz-jacketed, water cooled, high pressure mercury lamp (AH-6, General Electric). Even then it was not possible to make measurements between 273 and 239 mμ; and in contrast to the excellent reproducibility of measurements made above 300 mμ ($\pm 2\%$) it was not possible to delineate the Cotton effect below 300 mμ to a reliability of better than 20 per cent. The characteristic features of the profile were nevertheless repeatedly observed, and the profile shown represents an averaging of four series of observations. It should be emphasized that the variability was in the magnitude of the rotation and not on the location of the profile on the wave length axis.

An expanded picture of the region of the observed Cotton effect is shown in Fig. 10. The rotation reaches a maximum at 289 mμ and may be approaching a well near

[29] J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957).
[28] H. Boedtker, *J. Mol. Biol.* **2**, 171 (1960).

275 mμ, but the data are insufficient to assign a wave length to this Cotton effect. Upon complete denaturation at 95°, a marked change is noted in the region of the Cotton effect. The magnitude of the rotation drops substantially, but the Cotton effect does not seem to disappear; significantly, however, the wave length of maximum rotation shifts to the red by 4 mμ. Upon allowing the solution to cool, so that a substantial reformation of hydrogen-bonded base pairs occurs[1] the Cotton effect is seen to move partway back to its original position.

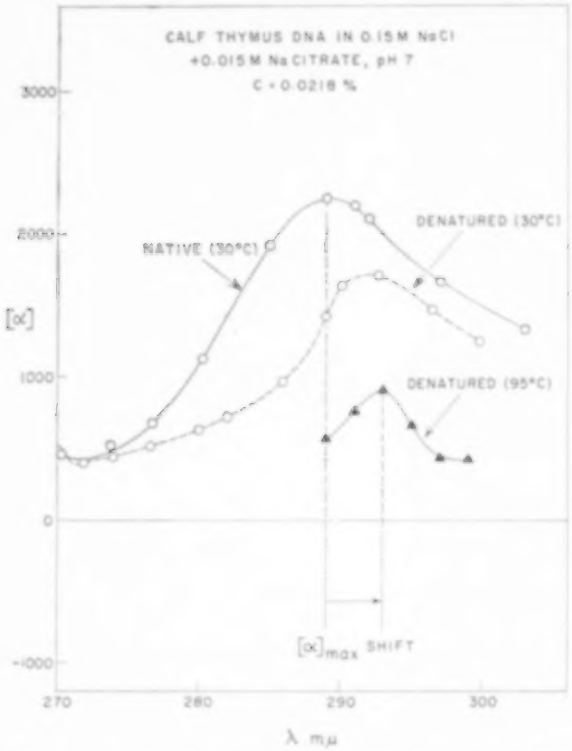

FIG. 10. Alterations in the Cotton effect with conformation of DNA.[18]

Since the observed Cotton effect is markedly displaced and diminished upon denaturation of the DNA, the transition involved is one that is probably accentuated by helical secondary structure. Both the red shift on denaturation and its location in a region where the absorption of DNA is very small suggest that this Cotton effect is due to an $n \rightarrow \pi^*$ transition. Because the Cotton effect is located considerably to the red of the λ_0 of DNA we may presume that other Cotton effects exist in the near-ultraviolet region, that some of them are of the $\pi \rightarrow \pi^*$ type, and that these may be of greater magnitude than the one that has been observed.

A fortunate consequence of our observation of a Cotton effect was that it first sensitized us to the existence of minor discontinuities in the absorption spectrum of polynucleotides. As in the case of poly A (cited above) we have observed diffuse fine structure on the long wave length side of the DNA absorption spectrum. This is

evident in Fig. 11, where it can be seen that the prominent shoulder which approximately coincides with the region of the observed Cotton effect disappears upon thermal denaturation of the DNA.

It is apparent from our preliminary exploration of the optical rotatory dispersion of DNA in the region of its near ultraviolet absorption that this region is rich with information which may ultimately prove useful in understanding the molecular structure of nucleic acids.

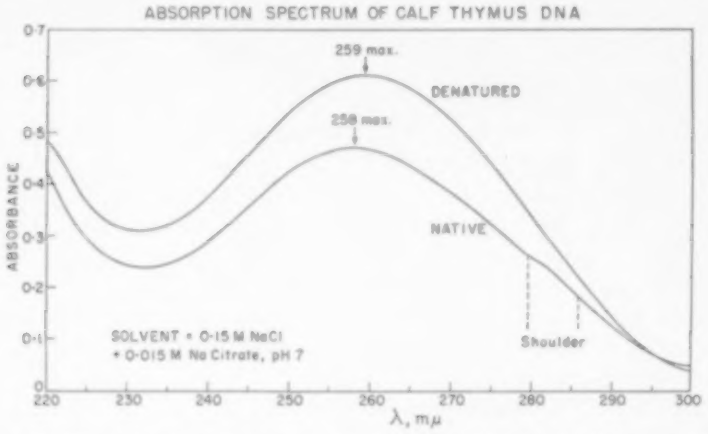

FIG. 11. Ultraviolet absorption spectra of native and heat denatured calf thymus DNA.[28]

Note added in proof: By suitably increasing the photosensitivity of the Rudolph spectropolarimeter we have now been able to extend the dispersion measurements through the near-ultraviolet absorption band of nucleic acids.[31] Native nucleic acids contain several closely spaced Cotton effects which have been sorted out into those due to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. The Cotton effect profiles for native RNA and DNA, and their dependence on secondary structure have features of similarity consistent with these two types of nucleic acids having a common element of helical structure. The differential sensitivity to denaturation which the two types of electronic transitions exhibit in both RNA and DNA is indicative of the differential influence of hydrogen bonding between bases and the stacking of base pairs in helical polynucleotides.

Acknowledgements—Various aspects of the experimental work emanating from this laboratory have been carried out in collaboration with Professor Paul Doty, Mrs. Elizabeth Klemperer, Mr. Robert Gorn and Mr. Richard Blake. I have profited also from enlightening discussions with Professor Michael Kasha and Dr. Helga Boedtker.

Much of the work described here was carried out with the support of grants from the United States Public Health Service, C-2170 and the American Heart Association.

[31] J. R. Fresco, A. M. Lesk, R. Gorn and P. Doty, *J. Amer. Chem. Soc.* In press (1961).

Tetrahedron, 1961, Vol. 13, pp. 198 to 207. Pergamon Press, Inc. Printed in Northern Ireland

STUDIES ON THE SECONDARY STRUCTURE OF NUCLEIC ACIDS BY MEANS OF OPTICAL ROTATION*

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THE success of employing optical rotation and rotatory dispersion methods for studying secondary structures of proteins and polypeptides has been well demonstrated in the literature,[1–3] as well as in reports given in this conference. Recently this technique also has been applied profitably to a study of the secondary structure of nucleic acids.[4–7] By the use of this method we have been able to obtain certain interesting information about nucleic acids *in vitro*, for it was found that the organic solvents formamide and dimethyl sulfoxide have profound effects on the secondary structure of DNA and RNA.‡ At times, it was necessary to supplement optical rotation data by measurements of ultraviolet absorption of these solutions or by analyses of the analytical ultracentrifuge.

The specific optical rotation, [α], of a solution containing equimolar amounts of deoxyribonucleotides and ribonucleotides is low (slightly levorotatory, depending on the isomers of the nucleotides used) with respect to that of the individual nucleotides, and it is essentially independent of temperature between 25° and 90°. On the other hand, the specific rotation of either the biosynthetic polynucleotides or the naturally occurring nucleic acids is strongly dextrorotatory (see Fig. 1 for pea microsomal RNA) and is greatly influenced by temperature. The decrease of dextrorotatory optical rotation of these nucleic acids has been shown to be related to the rupture of intramolecular hydrogen bonds and the transformation of helical conformation to random conformation[4–7] reminiscent of that of the polypeptides.[1,3]

The specific optical rotation of calf thymus DNA as a function of temperature is given in Fig. 2A, the results showing close agreement with those published by the Harvard group.[5] The specific rotation is only weakly dependent on temperature§ until near 80° in this particular buffer, then it drops abruptly within a narrow zone of temperature referred to as the "melting temperature" or "temperature of transition". The DNA, once so heated, gives a rotation vs. temperature curve different from

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† On leave from the University of California at Riverside.

‡ Abbreviations: DNA = deoxyribonucleic acid; RNA = ribonucleic acid.

§ The small increase of optical rotatory power before the transition temperature was also observed before.[5] No clear explanation has been suggested.

[1] E. R. Blout in *Optical Rotatory Dispersion* (Edited by C. Djerassi) p. 238. McGraw-Hill, New York (1959).
[2] J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957).
[3] E. R. Blout, P. Doty and J. T. Yang, *J. Amer. Chem. Soc.* **79**, 749 (1957).
[4] P. Doty, H. Boedtker, J. R. Fresco, B. D. Hall and R. Haselkorn, *Ann. N. Y. Acad. Sci.* **81**, 693 (1959).
[5] P. Doty, H. Boedtker, J. R. Fresco, R. Haselkorn and M. Litt, *Proc. Natl. Acad. Sci.* **45**, 482 (1959).
[6] J. R. Fresco, *Trans. N. Y. Acad. Sci.* Ser. II, **21**, 653 (1959).
[7] J. R. Freeco, *Fed. Proc.* **18**, 904 (1959).

that of non-heated DNA, indicating irreversibility in the process of loss of helical conformation. The once-heated DNA exhibits cyclic reproducibility of rotation vs. temperature curves between 25° and 90°, with the absence of any narrow zone of transition temperature. A comparable result was obtained from the measurement of the temperature dependence of optical density for the DNA solution (hypochromic effect) as shown in Fig. 2B. An increase of optical density of nucleic acid solutions had also been related to the loss of hydrogen bonding and helical structure. The once-heated

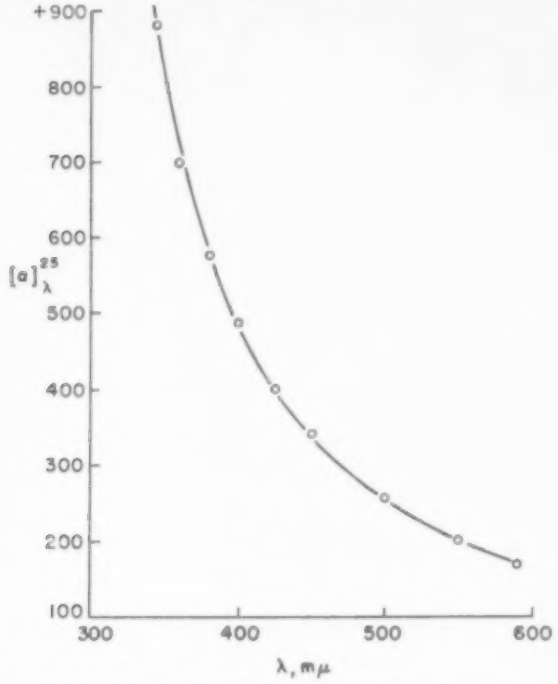
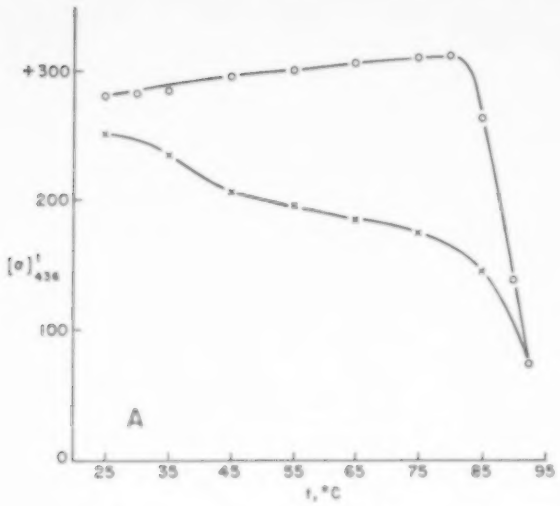


FIG. 1. Optical rotatory dispersion of pea microsomal RNA.

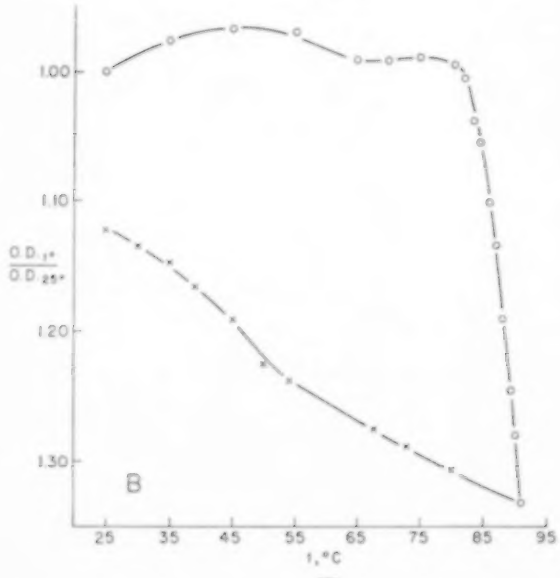
DNA again gave reproducible optical density vs. temperature curves cyclically, without a sharp transition zone of temperature, in contrast to the behavior of the unheated DNA. In this communication, any DNA will be referred to as denatured if it gives the rotation or optical density characteristics of that of the heated DNA (above 90° for 15 min).

In contrast to the DNA pattern, both the specific rotation vs. temperature and optical density vs. temperature curves of ribosomal RNA are smooth, without a narrow zone of transition temperature (Figs. 3A, B). Furthermore, they are essentially reversible, with only slight changes in magnitude and general shape of the curves.* In other words, although the RNA at 25° is likely to have a higher degree of hydrogen bonding and helical conformation than the RNA at 90°, the heated and unheated materials cannot be distinguished from each other by the dependence of their optical rotation or optical density on temperature; another measurement is required to identify the material with a history of heat treatment.

* Heating RNA in the buffer used for these measurements caused a drop of about 5% in the specific optical rotation after 20 min at 95°.



(A)



(B)

Fig. 2. Calf thymus DNA. Temperature dependence of (A) specific optical rotation, and (B) optical density at 260 mμ.

Upon examination in the ultracentrifuge, the pea microsomal RNA consists of two components with sedimentation coefficients of 27–28S and 17–18S (Fig. 4A).[8] After heat treatment at 85–95° for 10–15 minutes, it exhibits only a broad boundary of about 8–11S (Fig. 4B). Therefore, by analyses of ultracentrifuge, it is possible to detect RNA which has been once heated to a point where it has lost its intramolecular hydrogen bonding and original helical structure.

[8] P. O. P. Ts'o and R. Squires, *Fed. Proc.* **18**, 341 (1959).

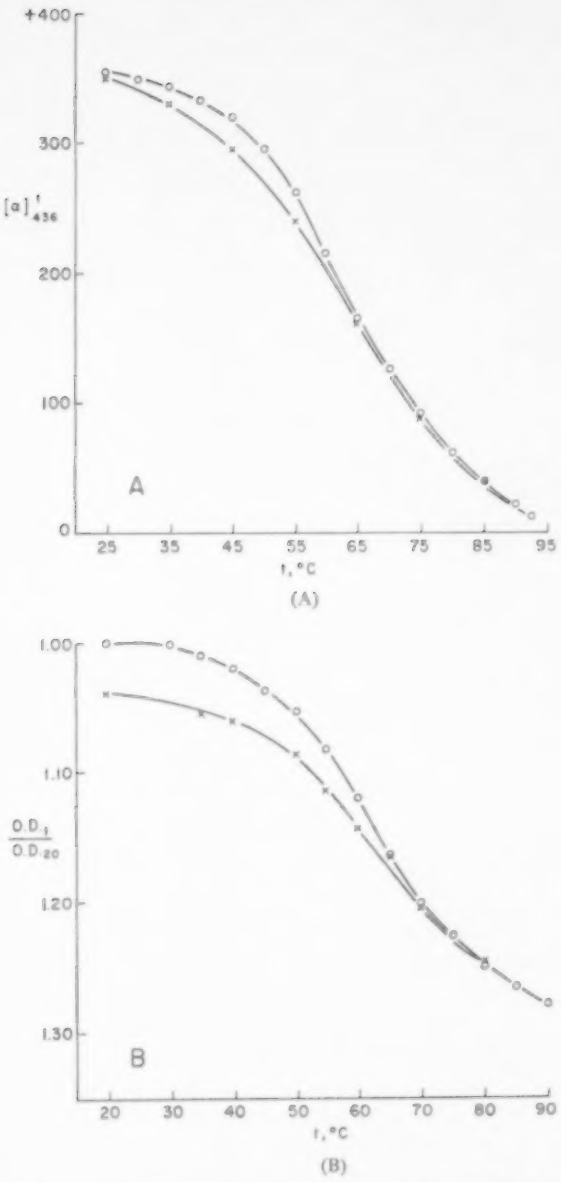


FIG. 3. Pea microsomal RNA. Temperature dependence of (A) specific optical rotation, and (B) optical density at 260 mμ.

The specific rotation vs. temperature curves given by DNA and RNA in formamide, dimethyl sulfoxide, and buffer are shown in Figs. 5A, B. The specific rotations of either DNA or RNA in these organic solvents are very low, or even negative, and essentially independent of temperature. These results suggest the loss of helical conformation of these nucleic acids while in solution in these organic solvents. The uncertainty

of other solvent effects on the optical rotation does exist, however, since free nucleotides in formamide or dimethyl sulfoxide tend to give more negative values than those obtained in aqueous solution.

After DNA and RNA had been dissolved in the two indicated organic solvents at room temperature, and without any heating, they were recovered by precipitation procedures and redissolved in aqueous buffer. It was apparent that the graphs of specific optical rotation vs. temperature and optical density vs. temperature from this recovered DNA (Figs. 6A, B) have the same characteristics as those of heat-denatured

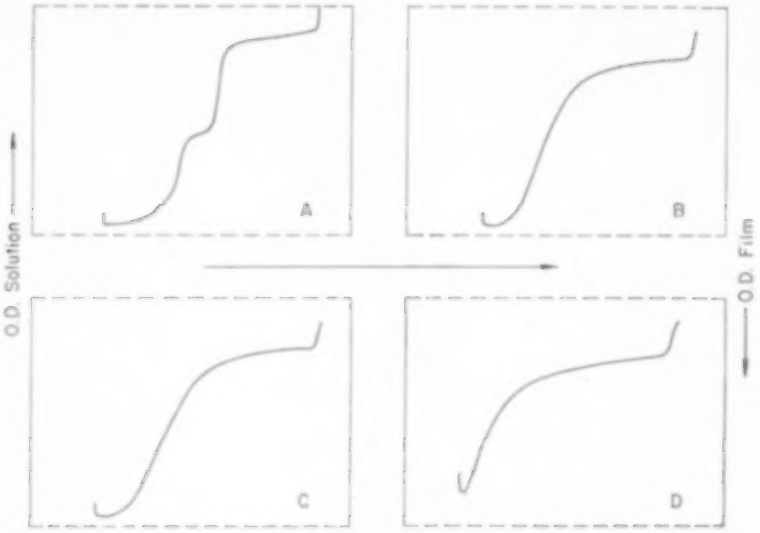


Fig. 4. Sedimentation patterns of pea microsomal RNA. (A) native, (B) heat denatured, (C) recovered from formamide, (D) recovered from dimethyl sulfoxide.

DNA, even though the DNA had never been heated higher than about 25° while in any solution. The data, therefore, does indicate that DNA in solutions of formamide and dimethyl sulfoxide at low temperatures loses most of its helical conformation just as if it had been in aqueous buffer at 90°.

Again in contrast to the behavior of DNA, the graphs of specific rotation vs. temperature (Fig. 7) and optical density vs. temperature for the recovered RNA are very similar to that of the original RNA. This is not surprising since the curves of heated and unheated RNA also show no variation. Ultracentrifuge analysis, however, shows the recovered RNA with a broad boundary with an average sedimentation coefficient of 8–10S (Fig. 4C, D). Thus the ultracentrifuge pattern of the RNA recovered from the organic solvents is similar to that of the heated RNA (Fig. 4B) but vastly different from that of the native, unheated RNA (Fig. 4A). These results again suggest that RNA in solution of these two organic solvents at room temperature loses most of its ordered secondary structure.

Titration of buffer solutions of the nucleic acids with formamide or dimethyl sulfoxide at a given temperature was followed by means of specific rotation measurements. DNA gave a sharp transition zone at 58 volume per cent (28 mole per cent*)

* Deviations in volume from ideality of up to 3% were not considered in making calculations of specific rotations.

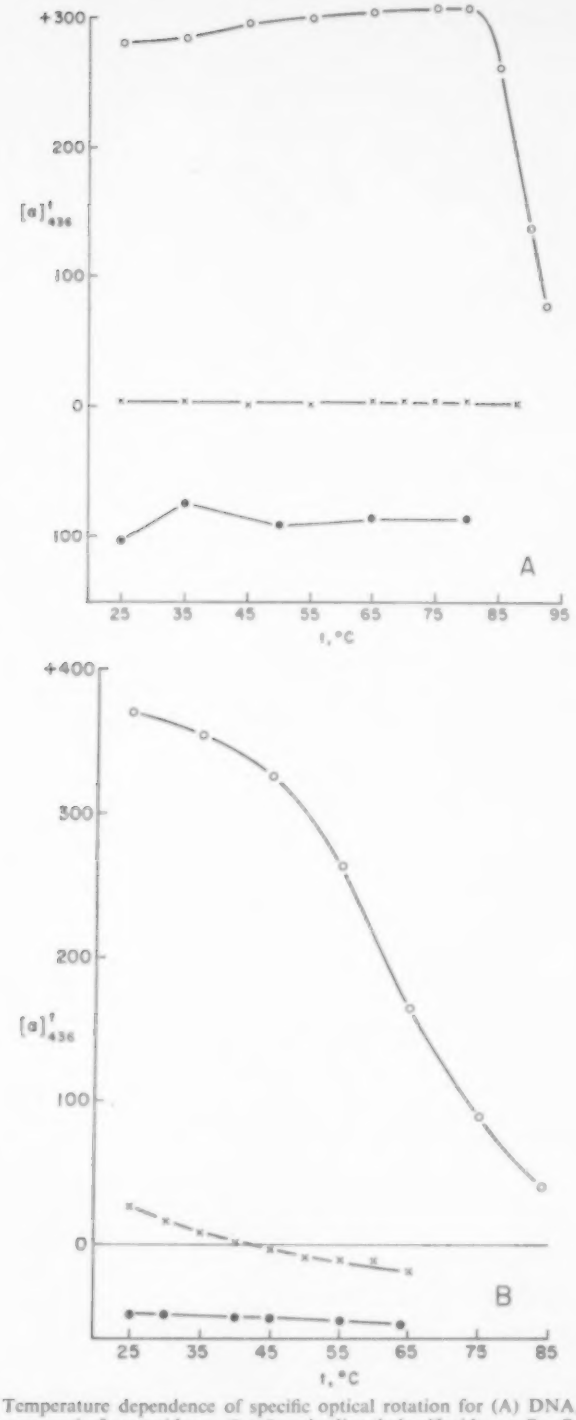


FIG. 5. Temperature dependence of specific optical rotation for (A) DNA, and (B) RNA; --×—×—, in formamide; —●—●—, in dimethyl sulfoxide; —○—○—, in buffer.

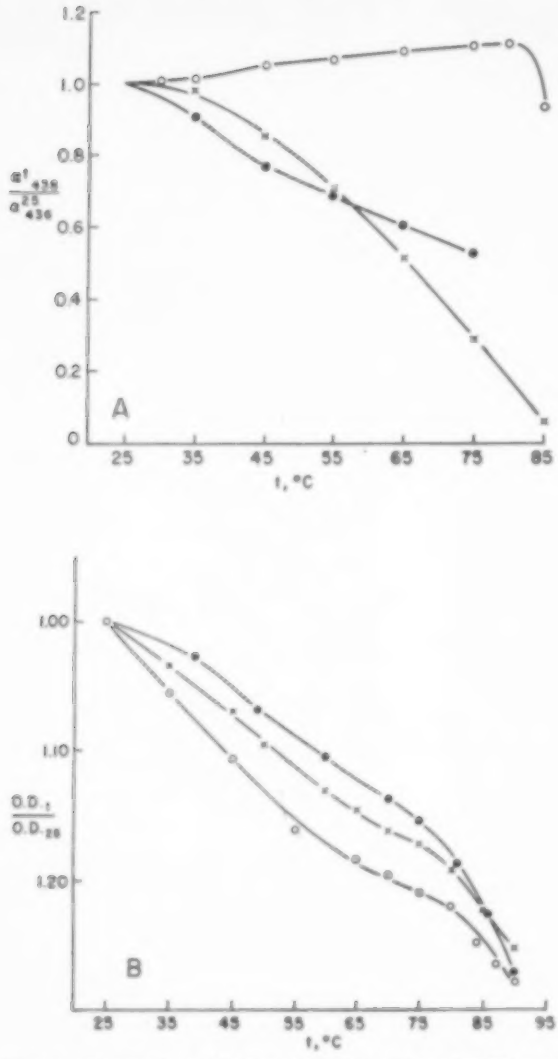


Fig. 6. Temperature dependence of (A) specific optical rotation and (B) optical density for calf thymus DNA. —○—○—, native in (A), heat denatured, in (B); —×—×—, formamide denatured; —●—●—, dimethyl sulfoxide denatured.

dimethyl sulfoxide and 70 volume per cent (51 mole per cent*) formamide (Fig. 8). RNA, on the other hand, gave a curve (Fig. 9) which, with appropriate coordinate scale, could be superimposed on that of the typical rotation vs. temperature curve. It should be mentioned that the DNA solution in an organic-aqueous mixture having the same composition as that for the transition zone exhibited a decrease of specific rotation with time which finally reached an equilibrium value. The kinetics of the denaturation process could then be studied.

The system of nucleic acid solutions in mixtures of buffer and formamide or

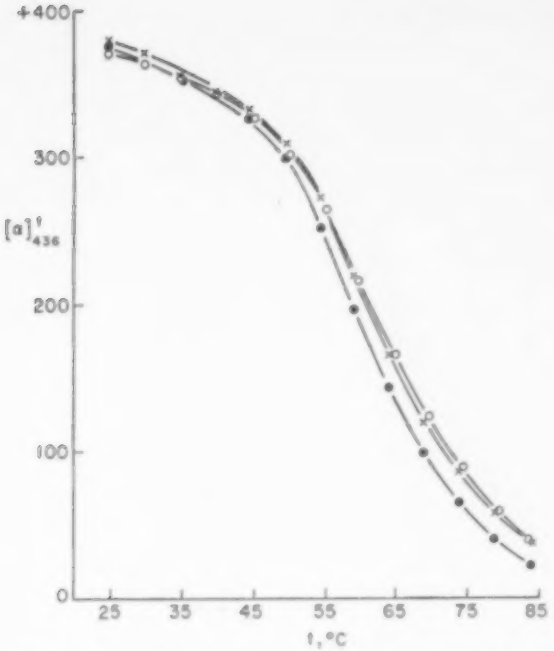


FIG. 7. Specific optical rotation vs. temperature for RNA denatured by: —○—○—, heat; —×—×—, formamide; —●—●—, dimethyl sulfoxide.

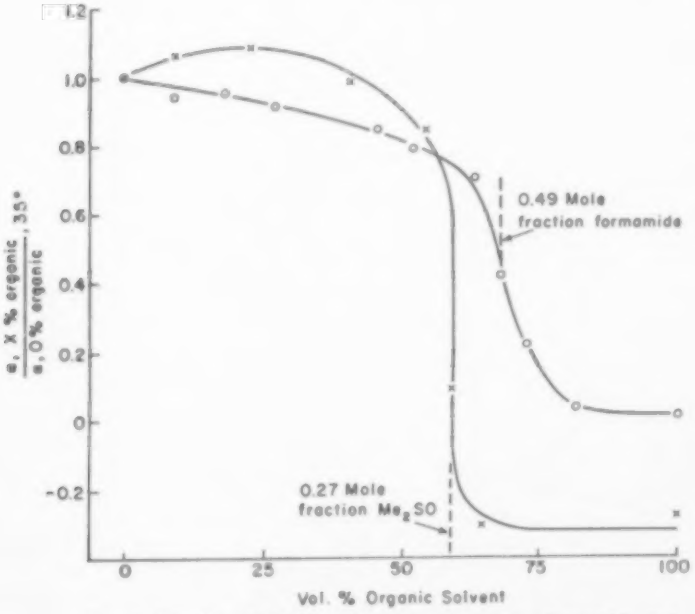


FIG. 8. Titration of DNA in buffer with: —○—○—, formamide, and —×—×—, dimethylsulfoxide.

dimethyl sulfoxide is reminiscent of that of the synthetic polypeptides such as poly-γ-benzyl glutamate in chloroform-dichloroacetic acid.[1,3] The helix-coil transformation in the case of the synthetic polypeptides is reversible, but that of the nucleic acids is not. Thus, a process of denaturation for both DNA and RNA is available at room temperature. In this process, the danger of unwanted chemical or enzymatic hydrolyses accompanying the rupture of hydrogen bonds is likely to be avoided. Information of the physical chemical properties of nucleic acids in these solvents (such as molecular weight) will be of great interest.

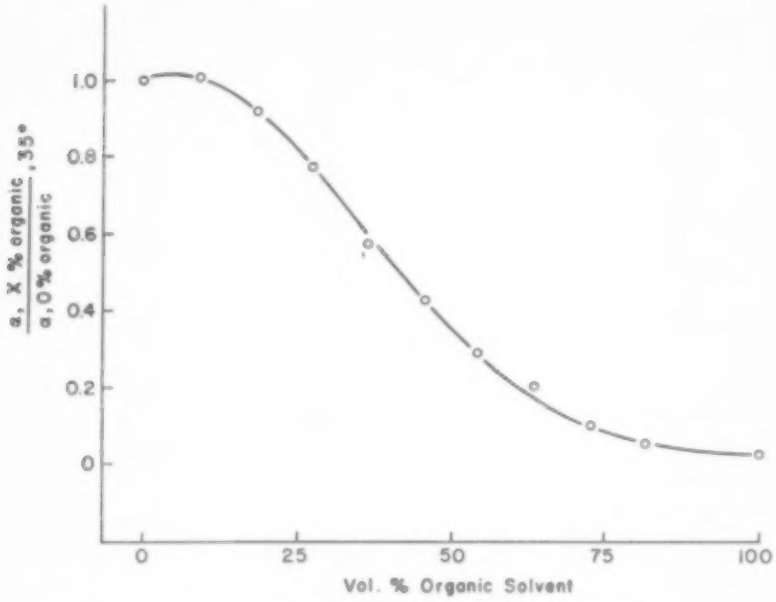


Fig. 9. Titration of RNA in buffer with formamide.

EXPERIMENTAL

Materials and reagents. All of the DNA used for the described experiments was calf thymus DNA purchased from Nutritional Biochemical Corporation, and some of its properties have previously been described.[9] Aqueous solutions of about 0·1% were prepared by mixing solvent and DNA in a slowly rotating flask for two days at room temp. Formamide solutions were prepared similarly, but the rate of solution was considerably higher. Dimethyl sulfoxide failed to show any significant effect on DNA after several days, even when the temp was raised to about 40°; solvation was effected by the addition of 20% by volume of water and rotating the mixture for 12 hr. Most of this water was removed by partial distillation at reduced pressure to about ⅓ original volume. DNA could be precipitated from formamide solution by the addition of 2 volumes of 100% ethanol and ½ volume absolute ethyl ether at 0°; or the formamide could be replaced directly by buffer solution by 3 dialyses over a period of 5 days in 20-fold amounts of buffer at 0°. The addition of 4 volumes of benzene precipitated DNA from dimethyl sulfoxide. Measured physical constants in buffer solution were $[\alpha]_D^{25}$ +135°, and $[\alpha]_{436}^{25}$ +260°.

Pea microsomal RNA was prepared according to the method described previously.[9] Immediately after preparation it was quick-frozen in buffer solution and stored at −50° until needed. Solutions in the 2 organic solvents could be prepared directly from the precipitated RNA (isolated from buffer solution by the addition of 3 volumes of cold ethanol and washing with absolute ethanol). Isolation from formamide solution was carried out by precipitation with 2 volumes of buffer and 6 volumes of

[9] W. F. Dove, F. A. Wallace and N. Davison, *Biochem. Biophys. Res. Com.* **1**, 312 (1959).

ethanol at ice temp. Last traces of solvent could be removed by repeated washing with absolute ethanol or ethyl ether; or by redissolving the precipitate in buffer and separating it a second time by alcohol dilution. The addition of 2 parts benzene and 2 parts ethanol (ethyl ether can be substituted for benzene) brought about precipitation of RNA from dimethyl sulfoxide. Measured physical constants were $[\alpha]_D^{25}$ +170°, and $[\alpha]_{436}^{25}$ +370°.

The organic solvents were 99% formamide (Eastman White Label), and practical grade dimethyl sulfoxide (Eastman) with a m.p. of about 18°. The aqueous solvent was an 0·1 M acetate buffer at pH 5·5 containing 0·1 M sodium chloride and 0·001 M magnesium chloride.

Instrumentation and methods of analysis. Measurements of optical rotations were made on a Rudolph Model 200S polarimeter with oscillating polarizer and xenon and mercury arc lamps. The polarimeter tubes were of unitized glass construction with water jacket, center-fill device, and quartz windows. The windows were sealed to the optically ground tube ends with an epoxy resin, Epocast 502. Although some problem of strain birefringence may have been inherent in the system, it was possible to reproduce zero readings at various temperatures with pure solvents in the polarimeter tubes. In most of the measurements it was possible to duplicate results on the instrument to better than ±2%.

Optical density measurements were made with a Beckman DK-2 ultraviolet spectrophotometer fitted with a modified temperature control device. Quartz cells were fitted with 20 mm immersion, standard taper thermometers for direct reading of solution temperatures. The cell compartment cover was adapted to pass the calibrated portion of the thermometer.

Analysis for the concentrations of nucleic acids was made indirectly by the colorimetric phosphate determination described by Allen[10], employing a Beckman DU spectrophotometer. Direct perchloric acid digestions were carried out only on the aqueous and formamide solutions. The thymus DNA was calculated to contain 9·30% phosphorus[11] and the microsamal RNA 9·09%.[12]

Analytical ultracentrifugation was performed in the Model E ultracentrifige, Spinco Division, Beckman Inc., with phase-plate schlieren optics and with ultraviolet absorption optics. The instrument was provided with a temperature control system. The absorption patterns were taken on commercial film with a 20 second exposure time on solutions of O.D.^{260} of 1·0 in 12 mm Kel-F cells. The photometric records were within the linear range of the characteristic curve of the film as shown by the trace of exponential aperture[13] in the counter balance cell in each run. The films were traced with a Double-Beam Recording Microdensitometer, Joyce Lobel Co., Newcastle upon Tyne, England. The direction of sedimentation of all the patterns is from left to right.

Acknowledgements—We wish to thank Professor James Bonner for his active support in this work, Dr. J. Vinograd and Prof. R. L. Sinsheimer for the use of certain instruments in their laboratories. The technical assistance of Mr. Christian Sander and Mrs. Ingelore Melvin is also gratefully acknowledged.

[10] R. J. L. Allen, *Biochem. J.* **34**, 858 (1940).
[11] E. Chargoff in *Nucleic Acids* (Edited by Chargoff and Davidson) Vol. II, p. 335. Academic Press, New York (1955).
[12] J. W. Wallace and P. O. P. Ts'o, unpublished data.
[13] E. Robkin, M. Meselson and J. Vinograd, *J. Amer. Chem. Soc.* **81**, 1305 (1958).

Tetrahedron, 1961, Vol. 13, pp. 208 to 218. Pergamon Press, Inc. Printed in Northern Ireland

ROTATORY DISPERSION STUDIES OF BIOLOGICALLY ACTIVE METALLOPORPHYRIN COMPOUNDS

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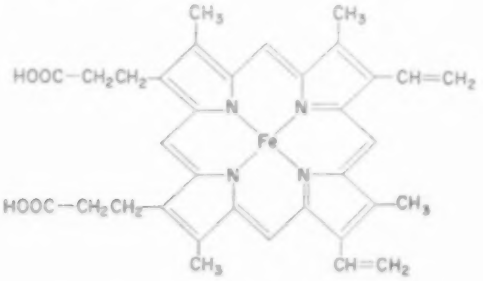
Metal atoms as asymmetric centers

THE recent application of rotatory dispersion studies in the elucidation of structures of biologically important molecules has involved consideration of two types of asymmetry; namely, the asymmetry of single carbon atoms, and the asymmetry due to the handedness of coiled macromolecules such as proteins and nucleic acids. Since many biomolecules contain metal atoms, and since such metal atoms can also give rise to asymmetry in the molecules, the presence of the metal atoms constitutes a third factor influencing the rotatory dispersion. Because the metal atoms frequently coincide with the site of biological activity, any experimental technique that focuses upon the metals should yield valuable clues about the structure of such molecules at important locations within them.

Many metallo-biological molecules contain the metal attached to a porphyrin, and it is with this class of compounds that the present discussion shall be concerned. The most frequent combination of metal and porphyrin that is encountered is the iron complex of protoporphyrin, called heme when the iron is in the +2 oxidation state; the structure of heme is as follows:



Examination of this structure reveals that it does not have a plane of symmetry outside the plane of the porphyrin. The plane of the porphyrin itself is therefore the only plane of symmetry that can be drawn through the iron atom in any molecule of which heme is a constituent.

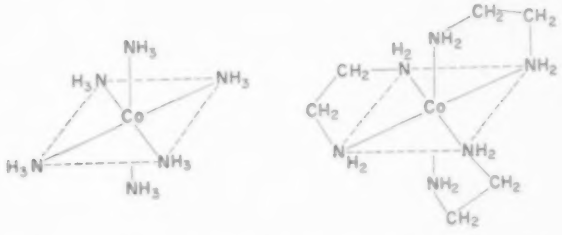
The iron atom is hexacovalent, and has its six bonds oriented toward the six corners of an octahedron. Four of the bonds are the iron-nitrogen bonds to the porphyrin; the other two bonds serve to attach the iron to the remainder of the biological molecule or to small molecules that have a biological function. It is the nature of these bonds perpendicular to the plane of the porphyrin that determines whether or not the iron atom is an asymmetric center of such a molecule. The two possibilities are exemplified

by a comparison of hematin and hemochrome[1] whose structures are as follows (only the nitrogen atoms of the porphyrin are shown):

Hematin Hemochrome

It is observed that hematin has a hydroxide ion attached to the iron on one side of the plane of the porphyrin and a water molecule on the other. The only possible symmetry plane has thus been destroyed, and the iron is asymmetric. In hemochrome, however, the substituents on both sides of the porphyrin plane are alike and, therefore, the iron atom is not asymmetric.

A second factor that determines the presence or absence of an asymmetric iron atom in such compounds can be understood by analogy with the following two complexes of cobalt:



The cobalt is not asymmetric in the hexammine complex, although it is asymmetric in the tris-ethylenediamine complex. In both compounds, the same molecules are attached to cobalt on both sides of the plane, but symmetry around the iron atom is prevented by chelation in the latter. It is thus possible to conclude that the iron atom will be an asymmetric center in all heme-containing compounds if (1) the two groups attached to the iron perpendicular to the porphyrin plane are different, or (2) the perpendicularly attached groups are also attached to porphyrin side chains thus producing a chelate structure such as that of tris-ethylenediamine-cobalt.

Cytochrome-c

Some of the known features of the structure of cytochrome-*c* are incorporated in Fig. 1. The iron atom is coordinated to protein through its fifth and sixth coordination positions, probably through histidine imidazole nitrogen atoms.[2] The protein is also attached at two places to protein side chains by the addition of two cysteine sulfhydryl groups to the double bonds of the two vinyl groups of protoporphyrin.[3] The partial

[1] R. Lemberg and J. W. Legge, *Hematin Compounds and Bile Pigments* pp. 169, 174. Interscience, New York (1949).

[2] H. Theorell and A. Akesson, *J. Amer. Chem. Soc.* **63**, 1818 (1941); E. Margoliash, *Nature, Lond*, **175**, 293 (1955). *Note added in proof:* It now appears more likely that only one of the protein donors is a histidine imidazole. E. Morgoliash, N. Frohwirt and E. Wiener, *Biochem. J.* **71**, 559 (1959).

[3] H. Theorell, *Biochem. Z.* **298**, 242 (1938); **301**, 201 (1939).

determination of the amino acid sequence[4] has revealed that the amino acids are not the same on opposite sides of the plane of the porphyrin. Thus both of the conditions which separately lead to an asymmetric iron atom are met by the cytochrome-*c* molecule.

It is expected, therefore, that cytochrome-*c* should exhibit a Cotton effect in the

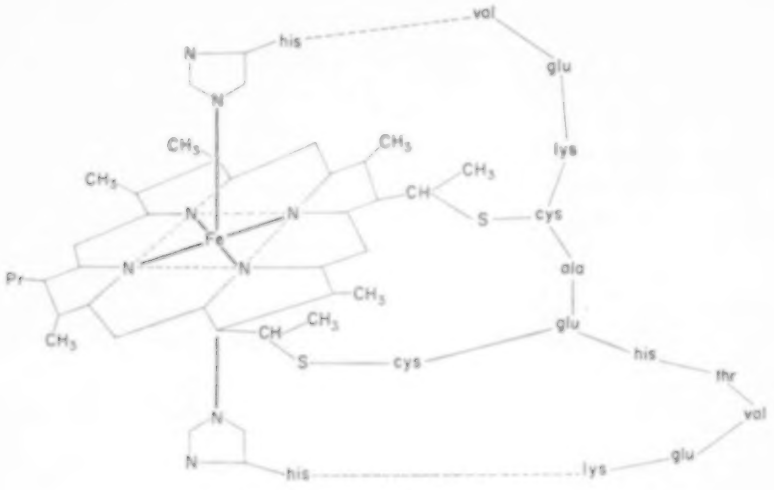


FIG. 1. Structure of cytochrome-*c*.

region in which the iron-to-nitrogen bonds absorb; i.e. around 530 mμ. Fig. 2 demonstrates that both the reduced and the oxidized forms of cytochrome-*c* do indeed show Cotton effects;[5] the optical rotation of the Fe(II) form is, however, more negative than that of the iron(III) form at all wavelengths. It is possible to keep on alternately oxidizing and reducing the same solution; the appropriate rotatory dispersion curve is reproduced in each instance. It is believed that this reproducibility indicates that the difference in the two dispersion curves may be attributed to the one-electron difference in the iron atom, rather than in any change induced in the protein by the redox reagents, since such changes could be expected to be less readily reversible.

The absolute values of the optical rotations of cytochrome-*c* at all wavelengths represent, of course, a summation of the effects of the iron asymmetry, the multiple carbon atom asymmetry, and the asymmetry due to the presumably partly helical configuration of the protein. The Cotton effect curve is thus superimposed upon another complicated curve, preventing the coincidence of a zero-rotation value with the absorption maximum, a phenomenon characteristic of the simple Cotton effect. That the Cotton effect can be observed at all is due to the fact that in the Cotton effect region the magnitude of rotation is much greater than anywhere else.

The metal atom asymmetry that is evident from an inspection of known structural features of cytochrome-*c* has thus been shown to manifest itself in the rotatory dispersion of the molecule. The possibility, therefore, presents itself that differences in the

[4] H. Tuppy and G. Bodo, *Monatsh.* **85**, 807, 1024, 1182 (1954); H. Tuppy and S. Paleus, *Acta Chem. Scand.* **9**, 353 (1955).
[5] G. L. Eichhorn and J. F. Cairns, *Nature, Lond.* **181**, 994 (1958).

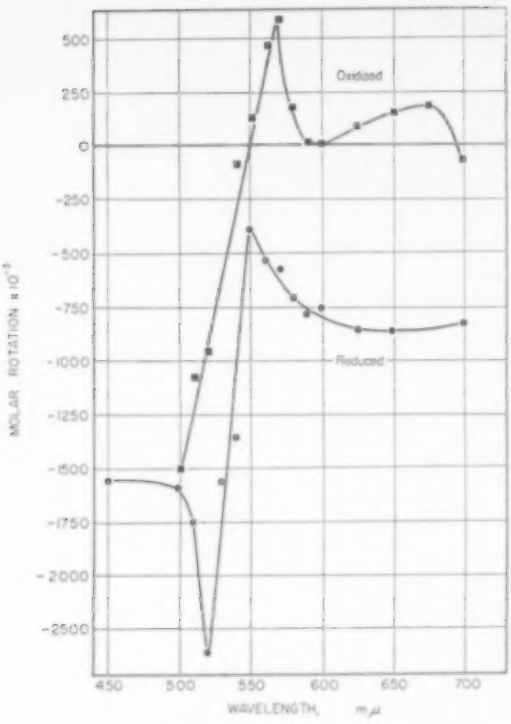


FIG. 2. Rotatory dispersion curves for cytochrome-*c*.

structures of hemoproteins could become correlated with differences in the rotatory dispersion curves, so that this technique could be developed for the study of hemoprotein structure as Djerassi has developed it for the study of the structure of steroids.

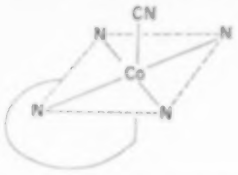
Attempts to obtain such correlation directly from the hemoproteins are complicated by the large gaps in our knowledge of the structure of these substances, as well as by the uncertainty about changes in the protein that might accompany changes in the iron coordination sphere. Vitamin B_{12} can, however, serve as a useful model for the hemoproteins, because the position of all of the atoms in vitamin B_{12} has been explicitly determined,[6] and changes in the coordination sphere of the metal are easily brought about.

Vitamin B_{12}

Vitamin B_{12}, or cyanocobalamin, is unlike the hemoproteins in that it contains cobalt, rather than iron, in that the porphyrin lacks a carbon atom and is partly saturated, as well as in that no protein is linked to the metal (see Fig. 3). It resembles the hemoproteins in that the metal is attached to four nitrogen atoms in a porphyrin-like structure, and in that one of the remaining two coordination positions of the hexacovalent cobalt is held by an electron donor which, in turn, is attached to the side

[6] B. Bonnett, J. R. Cannon, A. W. Johnson, I. Sutherland, A. R. Todd, and E. Lest. Smith, *Nature, Lond.* **176**, 328 (1955); D. C. Hodgkin, J. Pickworth, J. H. Robertson, K. N. Trueblood, R. J. Prosen and J. G. White, *Ibid.* **176**, 325 (1955).

chain of the pseudoporphyrin. The sixth position in cyanocobalamin is occupied by a cyanide ion, so that the configuration around the cobalt can be represented as follows:



Cyanocobalamin, like the hemoproteins, has asymmetry in addition to that around the metal; there are fifteen asymmetric carbon atoms in the molecule. These should contribute to the optical activity, but should not themselves exhibit a Cotton effect in the visible.

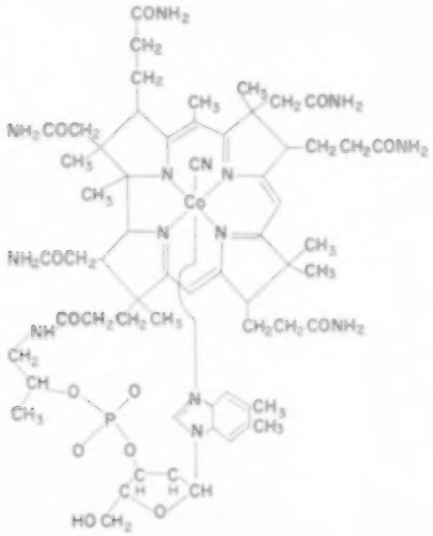


FIG. 3. Structure of vitamin B_{12}.

Figure 4 represents the spectrum and rotatory dispersion of cyanocobalamin.[7] The relative position of the two curves leads to the supposition that the optical activity may be related to the shoulder of the spectrum at 520 mμ rather than the maximum at 550 mμ.

Treatment of cyanocobalamin with weak acid brings about the substitution of the coordinated cyanide ion by a water molecule;[8] a cobalt–carbon bond is thus replaced by a cobalt–oxygen bond. It is significant that such a change in the cobalt coordination sphere, even though it results in a minor spectral change, does not cause any detectable difference at all in the rotatory dispersion curve. Evidently, if it is possible to generalize from this observation, rotatory dispersion curves are not likely to be useful in the study of such substitutions in hemoproteins.

When cyanocobalamin is made strongly acid, the bond from cobalt to the benzimidazole, in addition to that to cyanide, is broken.[8] The spectrum and rotatory

[7] G. L. Eichhorn and M. A. Stevan, unpublished observations.
[8] P. George, D. H. Irvine and S. C. Glauser, *Ann. N.Y. Acad. Sci.* **88**, 407 (1960).

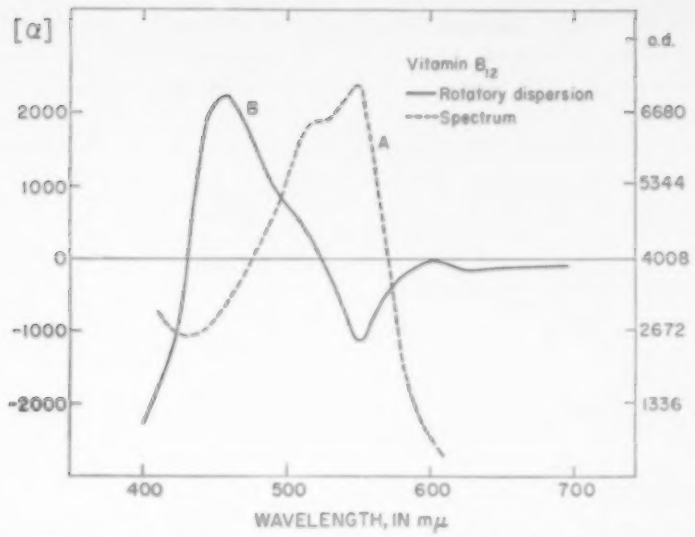


FIG. 4. Native vitamin B_{12}: A, spectrum of 0·01% solution; B, rotatory dispersion curve of 0·05% solution.

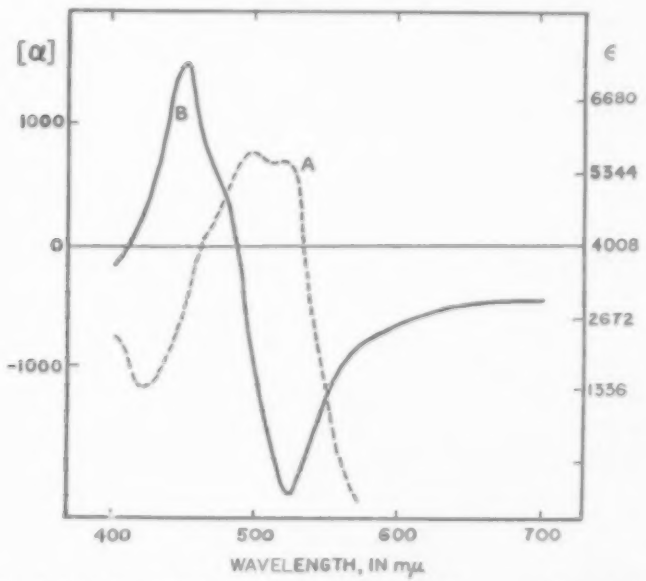


FIG. 5. Strongly acidified vitamin B_{12}: A, spectrum; B, rotatory dispersion.

dispersion of the resulting compound, which contains the originally chelated group now attached only to the side chain of the pseudoporphyrin, are shown in Fig. 5.[7] It thus appears that, although the substitution of one small molecule for another in the cobalt coordination sphere does not affect the rotation, a change in the shape of the molecule, such as is produced when the benzimidazole link is broken, affects the rotation significantly.

When the cobalt atom is reduced from its oxidation state of +3 in the unaltered vitamin to +2, the spectrum is changed very considerably, but the rotatory dispersion curve is displaced relatively little, as shown in Fig. 6.[7] Apparently the oxidation state of the metal, in this instance as well as in cytochrome-*c*, has not greatly affected the rotation. When, however, the reduced cobalt is reoxidized to the +3 state, the optical rotation becomes low at all points. It has been observed that when the cobalt is reoxidized in this manner, the vitamin B_{12} molecule dimerizes;[9] the present phenomenon is interpreted as indicating that the loss of rotation results from internal compensation of the activities of two cobalt atoms in the same molecule. Treatment of the reoxidized

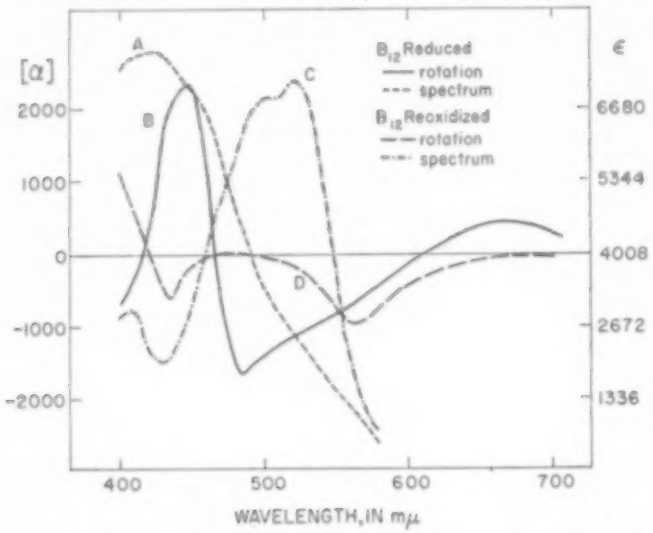


FIG. 6. Reduced vitamin B_{12}: A, spectrum; B, rotatory dispersion. Reoxidized vitamin B_{12}: C, spectrum; D, rotatory dispersion.

molecule with hydroxide ion, cysteine, or human serum albumin results in the reappearance of strongly rotating substances; in each case, the rotatory dispersion curve looks somewhat like a mirror image of the curve of the reduced vitamin treated in the same manner. (Fig. 7 shows the effect with HSA.[7]). It is possible to explain this phenomenon by supposing that the three reagents attack the oxidized dimer (B) on the side of the cobalt atom opposite that to which cyanide was originally attached. The benzimidazole group is then forced to swing around and coordinate to the cobalt in such a manner so as to produce a molecule (C) that is essentially an antipode of the intact vitamin (A); this hypothesis is depicted in Fig. 8.

It is possible alternatively to explain this "mirror image" effect by supposing that it merely accompanies the shift in the spectrum. The former explanation is preferred, because of the large differences in the dispersion curves of the reoxidized vitamin (e.g., Fig. 7B) and the original cyanocobalamin (Fig. 1B); treatment of the latter with hydroxide ion or cysteine produces rotatory dispersion curves that are identical to Fig. 4B. The evidence for the inversion mechanism is, however, not conclusive.

[9] B. Jaselkis and H. Diehl, *J. Amer. Chem. Soc.* **80**, 2147 (1958).

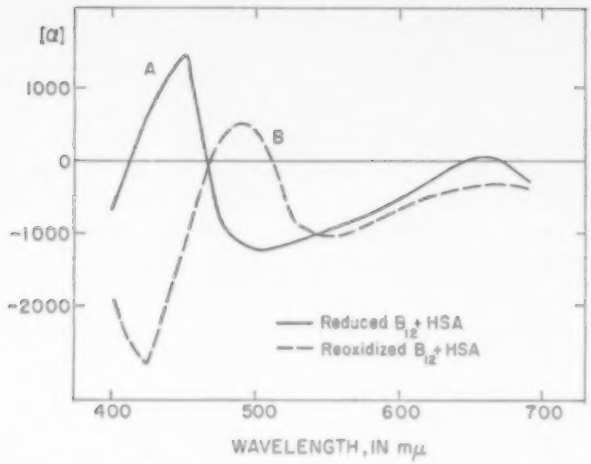


FIG. 7. Rotatory dispersion curves of: A, reduced vitamin B_{12} and HSA; B, reoxidized vitamin B_{12} and HSA.

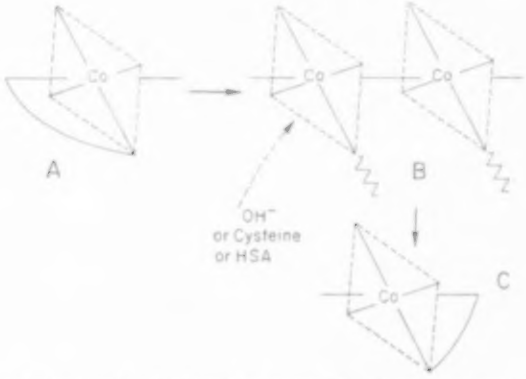


FIG. 8. Proposed mechanism for vitamin B_{12} substitution reactions.

Application to structural studies on hemoproteins

Hemoglobin and catalase. It had been hoped that rotatory dispersion measurements could be utilized in the solution of such problems as the nature of the groups attached to the iron atom in hemoglobin. Such an application appears improbable in view of the fact that rotatory dispersion cannot differentiate between a cobalt–carbon and a cobalt–oxygen bond in vitamin B_{12}. The results with vitamin B_{12} lead to the expectation that differences in the rotatory dispersion curves reflect extensive changes in the molecular configuration surrounding the metal. It seems reasonable, therefore, that rotatory dispersion can be profitably applied to structural studies of hemoproteins that contain more than one molecule of heme per molecule of intact hemoprotein. A comparison of the rotatory dispersion of the intact molecule with that of a molecule that has been cleaved represents a comparison of the symmetries around the heme group in the intact and divided molecules, and can thus lead to an understanding of the relationship between heme and proteins in such molecules. Both hemoglobin and catalase contain

four hemes; the following accounts demonstrate the use of rotatory dispersion measurements in structural studies with each of these substances.

The hemoglobin molecule contains two pairs of polypeptide chains; the chains of each pair are identical but different from the chains of the other pair.[10] Each polypeptide chain is associated with one heme group. It has been shown that the intact hemoglobin molecule containing four hemes can be split into half molecules, each retaining two hemes. Two methods for producing half molecules, though not necessarily the same halves, are the addition of base[11] and the addition of urea.[12] The

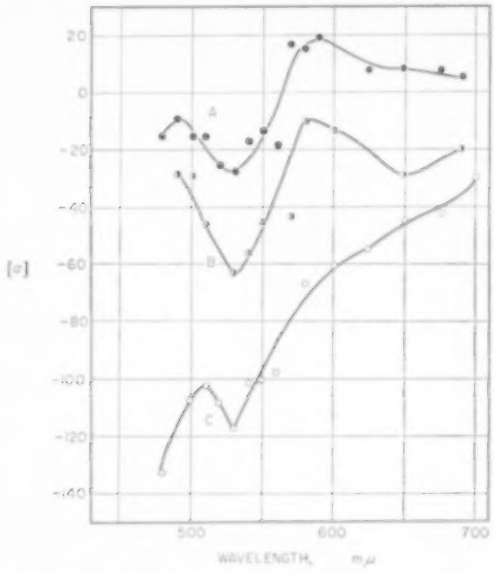


FIG. 9. Rotatory dispersion curves of hemoglobin: A, pH 7; B, pH 11; C, in 8-molar urea, pH 7.

rotatory dispersion curves of a sample of horse hemoglobin before treatment,[13] and after treatment with base, or with 8-molar urea, are shown in Fig. 9.[14] It can be seen that the Cotton effect present in whole hemoglobin is retained in the pH 11 half-molecules; the shape of the curve is essentially unaltered, although the negative rotation is enhanced at all wave lengths. It is concluded that the half-molecules at pH 11 are slightly denatured, but that the heme-protein relationship remains unchanged.

In 8-molar urea, on the other hand, there is a much more drastic increase in the negative rotation values, and the Cotton effect becomes less significant. It appears that 8-molar urea produces much more extensive denaturation than alkali, and possibly the symmetry around the heme is increased. At any rate, the half-molecules that are formed by the two different treatments are significantly different from each other.

[10] H. S. Rhinesmith, W. A. Schroeder and L. Pauling, *J. Amer. Chem. Soc.* **79**, 4682 (1957); H. S. Rhinesmith, W. A. Schroeder and N. Martin *Ibid.* **80**, 3358 (1958).
[11] V. Hasserodt and J. Vinograd, *Proc. Nat. Acad. Sci.* **45**, 12 (1959).
[12] H. Wu and E. F. Yang, *Chinese J. Physiol.* **6**, 51 (1932); J. Steinhardt, *J. Biol. Chem.* **123**, 543 (1938); F. J. Gutter, H. A. Sober and E. A. Peterson, *Arch. Biochim. Biophys.* **71**, 342 (1957).
[13] The shape of the hemoglobin curve varies somewhat with its source; however, the described changes with alkali or with urea are always comparable. P. Doty *et al.* have independently obtained similar curves.
[14] J. Cairns, Thesis, Georgetown University (1957).

The results with catalase are somewhat more interesting. Tanford[15] has demonstrated that catalase is broken up into quarter molecules at pH 11, each quarter molecule containing one heme group. The spectra[16] of catalase at pH 7·5, where it is whole, and at pH 11, where it is quartered, are compared in Fig. 10, and it can be seen that they do not differ in any important manner, both exhibiting a maximum at approximately 620 mμ. The rotatory dispersion curves, shown in Fig. 11, are, on the other

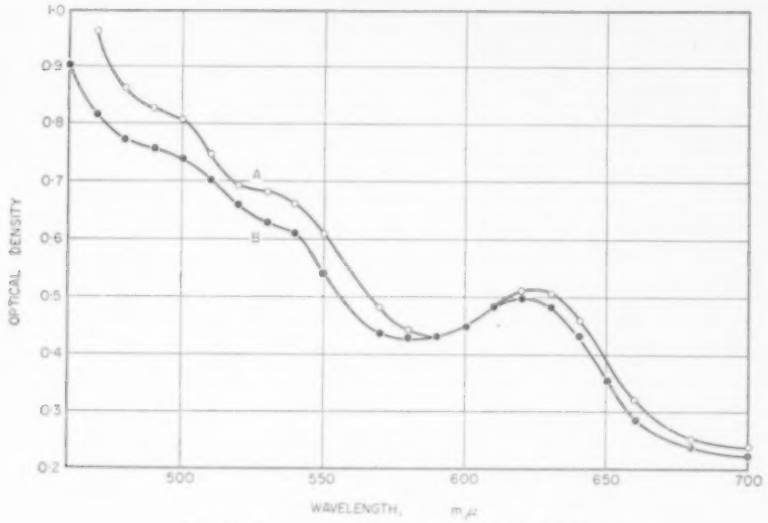


FIG. 10. Spectra of catalase: A, pH 7·5; B, pH 11·0.

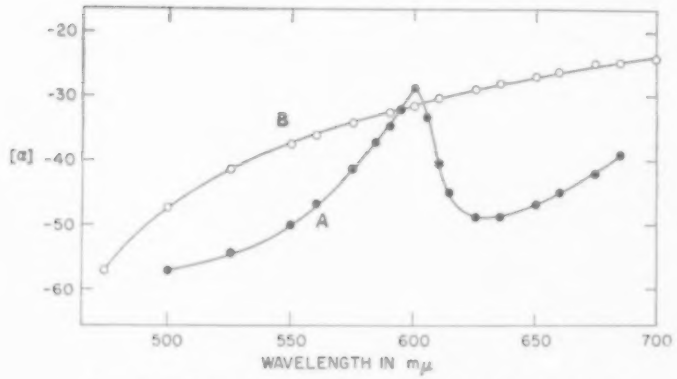
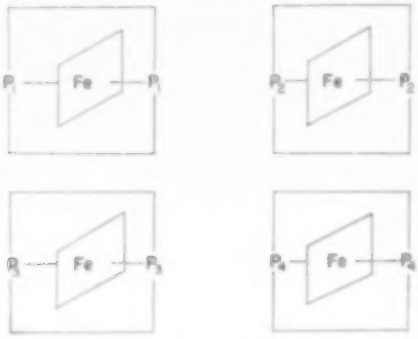


FIG. 11. Rotatory dispersion of catalase: A, pH 7·5; B, pH 11·0.

hand, exceedingly different;[16] at pH 7·5 the curve has a Cotton effect in the anticipated region, whereas at pH 11 the dispersion is "simple", leading to a value of λ_c of approximately 210 mμ. This difference is interpreted by the hypothesis that each quarter molecule of catalase is surrounded by a polypeptide chain in an essentially symmetrical manner, so that the iron atom is not an asymmetric center. However,

[15] C. Tanford and R. E. Lovrien, *Abstracts, 135th Meeting, Amer. Chem. Soc.* p. 14C. Boston, April (1959).
[16] A. Osbahr, Thesis, Georgetown University (1960).

association of the four quarters in the intact catalase molecule produces asymmetry. One way in which this can happen is shown below:



According to this model, the iron atom in each individual quarter is symmetrically surrounded; in the whole molecule, however, there is only part of one polypeptide chain on one side of the plane of the porphyrin, but on the other side there is not only the other half of the same polypeptide chain but also an entire quarter-molecule containing another heme.

Blout[17] has shown that organic dyes bound to helical proteins produce a Cotton effect, whereas the same dyes bound to randomly coiled proteins do not. It is believed that the observations on catalase should probably not be explained in this manner, if the Cotton effect of the helical protein-dye complexes is due to a stacking[18] of the dye molecules, a circumstance that does not seem to apply in catalase.[19]

Conclusions

The presence of a metalloporphyrin in a molecule is associated with a Cotton effect in the visible region of the spectrum, when the metal constitutes an asymmetric center of the molecule. The rotatory dispersion appears to be insensitive to simple substitution reactions in the metal coordination sphere, but it is greatly influenced by such reactions when they affect the gross molecular structure. These considerations are useful in the elucidation of the relationship of the metalloporphyrins in these substances to the other parts of the molecule.

[17] E. R. Blout and L. Stryer, *Proc. Natl. Acad. Sci.* **45**, 1591 (1959).
[18] D. F. Bradley and G. Felsenfeld, in press.
[19] *Note added in proof:* Experiments carried out since this paper was written lead to the conclusion that the rotatory dispersion of catalase is, indeed, a manifestation of the Blout–Stryer effect.

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LIQUID-CRYSTALLINE STRUCTURES IN POLYPEPTIDE SOLUTIONS

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VOL. 13 1961

INTRODUCTION

IN this paper the remarkable properties, including the very high optical rotatory power, of a liquid crystalline phase which is formed by certain polypeptide solutions will be discussed.

Although liquid crystals have attracted much interest since their discovery in 1888[1] (a recent review article contained as many as 475 references)[2] many physical chemists seem to be unfamiliar with the subject. It may therefore be helpful to state briefly what is meant by this rather unsatisfactory term.

Most crystals pass directly at their melting point from the crystalline state to an isotropic liquid, but many crystalline solids pass through one or more mesophases which, though birefringent, are more or less fluid. These mesophases have various degrees of organisation but most can be described by one or other of two terms introduced by Friedel: smectic and nematic.[3]

In the smectic structure the molecules are arranged in layers, which are themselves arranged in families of closed surfaces known as Dupin cyclides, which give rise to focal conics which are visible in a polarization microscope and can be used for the identification of such structures. These smectic structures and the microscopic patterns associated with them have been adequately described elsewhere.[3,4] The nematic structure, which is the only one of the two classes with which this paper is concerned, has a lower degree of organization that the smectic; there are no layers, but the molecules are arranged with their long axes parallel, while being free to move relative to one another in the direction of these axes. Friedel also described a third, though less familiar, type of structure, met with chiefly in esters and ethers of cholesterol, which is related to the nematic structure but which has a higher degree of organization. In what follows it will be found that these last have much in common with those which have been found to occur in certain polypeptide solutions.

A given material may form one or several mesophases each of which is stable between two well defined and reproducible transition temperatures and the temperature range over which any one is stable may cover a considerable number of degrees. These mesomorphic forms are not only produced on heating certain solids but with some substances (e.g. soaps, and the polypeptides to be discussed in what follows) on adding a limited amount of a suitable solvent. In these cases the mesophase may be stable over a considerable and reproducible range of *concentration*, which will, however, depend on the temperature. Such mesophases, whether formed by changes of temperature or addition of solvent, are generally called liquid crystals or liquid crystalline

[1] F. Reinitzer, *Monatsh. Chem.* **9**, 421 (1888).
[2] G. H. Brown and W. G. Shaw, *Chem. Rev.* **57**, 1050 (1957).
[3] G. Friedel, *Ann. Physique* **18**, 273 (1922).
[4] N. H. Hartshorne and A. Stuart, *Crystals and the Polarization Microscope* (3rd Ed.). London (1960).

structures. The term "liquid crystals" is somewhat inadequate and slightly misleading, but, as it is established by long usage, it will be used in this paper. It should be stressed that these mesophases are definite forms of matter, a knowledge of which is essential for the full understanding of any material which can give rise to them.

The liquid-crystalline phase in poly-γ-benzyl-L-glutamate

Solutions of poly-γ-benzyl-L-glutamate (PBLG) in certain solvents (e.g. dioxan, methylene chloride, chloroform, *m*-cresol) when above a certain concentration, A, separate into two liquid phases, the phase of higher concentration being spontaneously birefringent.[5,5a] If the concentration is above a still higher concentration, B, only the birefringent phase will exist. The values of A and B in a particular solvent depend on the molecular weight of the polymer, the birefringent phase appearing at a lower concentration with a higher molecular weight. The birefringent phase often first separates in the form of liquid spherulites, that is to say, birefringent liquid spheres showing a black maltese cross when observed between crossed polars.* On cooling or concentrating the solution these spherulites grow in size and eventually coalesce to form a continuous phase. Alternatively if the continuous phase is disturbed, long viscous streamers or filaments may separate from it which, as the disturbance ceases, break up into spherulites. The size and stability of the spherulites is very variable, depending as it does on numerous factors (density of solvent, interfacial tension, concentration, viscosity, purity, etc.).

The black maltese cross together with the sign of the birefringence indicate that the major refractive index, which here means the molecule, lies tangentially rather than radially. Much more remarkable, however, is that both the spherulite and the continuous birefringent phase show equi-spaced alternate dark and light lines (Fig. 1) which are visible even in natural light. The periodicity of these lines, S, depends on concentration, solvent, temperature, etc., but has a reproducible value, for any one set of conditions and may be as large as 100 μ or too small for the microscope to resolve. The periodicity is independent of the arrangement of the lines (e.g. whether parallel lines or concentric curves are observed), the optical system, or the shape of the vessel. Between crossed polars periodic retardation colours are seen, and, by using a quartz-wedge it can be shown that the retardation plotted against distance in a direction at right angles to the lines has an oscillating value and forms a smooth continuous curve.

Figs. 2 and 3 show a particularly large and regular spherulite. It will be seen that it shows one radial line of dislocation, or rather "disinclination", as Frank has called such lines in liquid crystals.[6] Each spherulite contains one, and only one, such radial line. The spherulites therefore, although fluid, have polarity. Similar lines. of disinclination are also to be seen in the continuous phase.

This description of the spherulites at once makes it apparent that we are concerned with a liquid crystalline structure of unusual interest. To understand the nature of this structure it will be simpler to consider the patterns that arise in a flat rectangular cell, say 1 cm square and 1 mm deep. After filling the cell families of equi-spaced lines gradually appear, those near the edge being parallel to the sides of the cell. Further

* The word polar is used for either a polarizer or an analyser.

[5] C. Robinson, *Trans. Faraday Soc.* **52**, 571 (1955).
[5a] A. Elliott and E. J. Ambrose, *Disc. Faraday Soc.* **9**, 246 (1950).
[6] F. C. Frank, *Disc. Faraday Soc.* **25**, 29 (1958).

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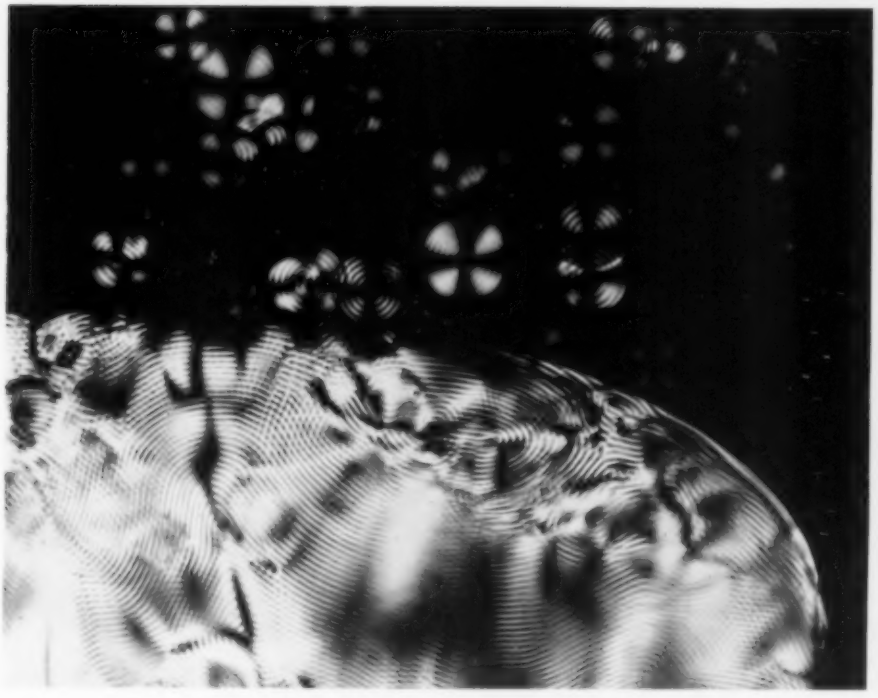

FIG. 1. Two phase solution of PBLG in dioxan; S = 14 μ. Crossed polars.

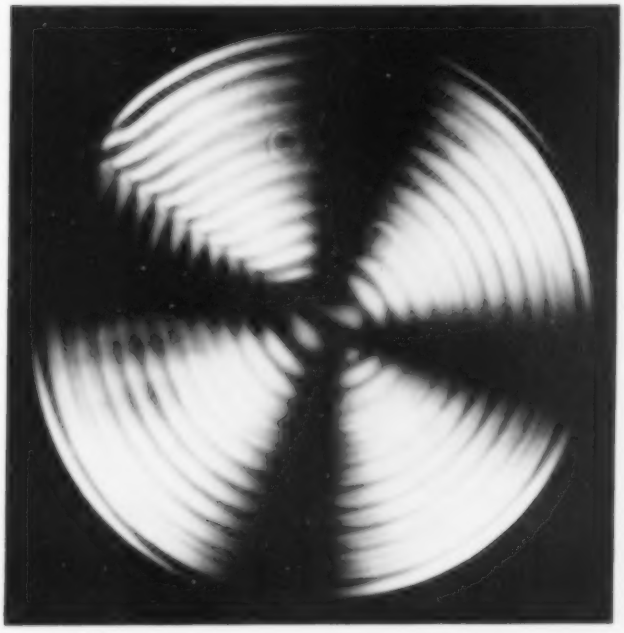

FIG. 2. Spherulite is solution of PBLG in methylene chloride. S = 15 μ. Crossed polars.

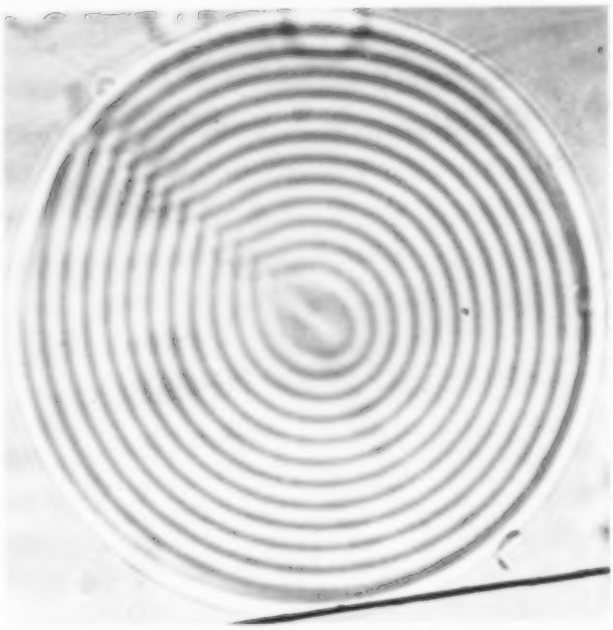

FIG. 3. The same spherulite as in Fig. 2, but in natural light.

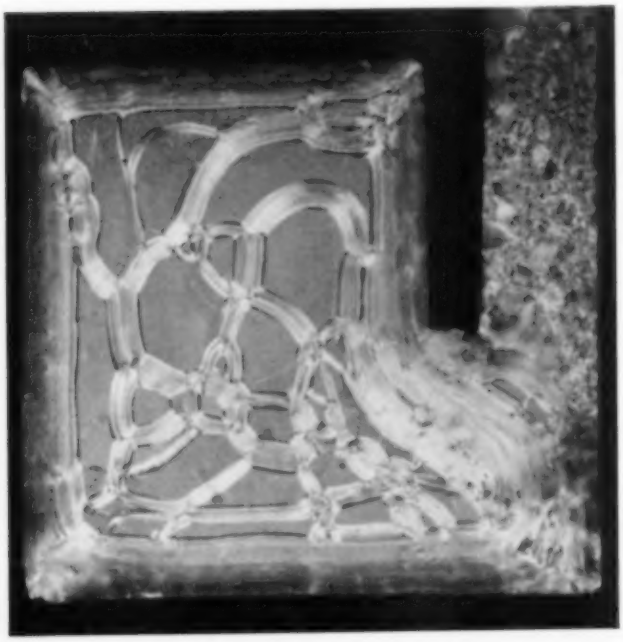

FIG. 4. Uniform areas in rectangular cell. (1 cm × 1 cm × 0·1 cm). Magnification too low to resolve the periodic lines. PBLG in methylene chloride.

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FIG. 5. Uniform areas with surrounding system of periodic *fines*. Solution of PBLG in dioxan in circular cell, 1 mm deep. The line system is largely concentric with the circumference of the cell, a small arc of which can be seen in the top left-hand corner. S = 21 μ. Polars at 45°, ↖↗. The uniform areas appeared bright blue.

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from the sides the cell becomes filled with groups of equidistant lines grouped in complicated and irregular patterns. These groups of lines are seen to undergo gradual rearrangement towards a simpler and more uniform pattern. Sometimes sudden rearrangements take place, the movements being somewhat similar to those of bubbles coalescing in a foam. As time goes on small, clear, polygonal areas appear which contain no lines but which are framed in continuous lines which lie parallel to the sides of the polygon (the corners of the polygon may be rounded and in some cases the whole of a side may be curved) (Figs. 4 and 5). These uniform areas tend to grow in size, neighbouring areas coalescing to form a larger area. The appearance of the parallel lines at once strongly suggests that we are looking at layers seen edge on, and, if we imagine the

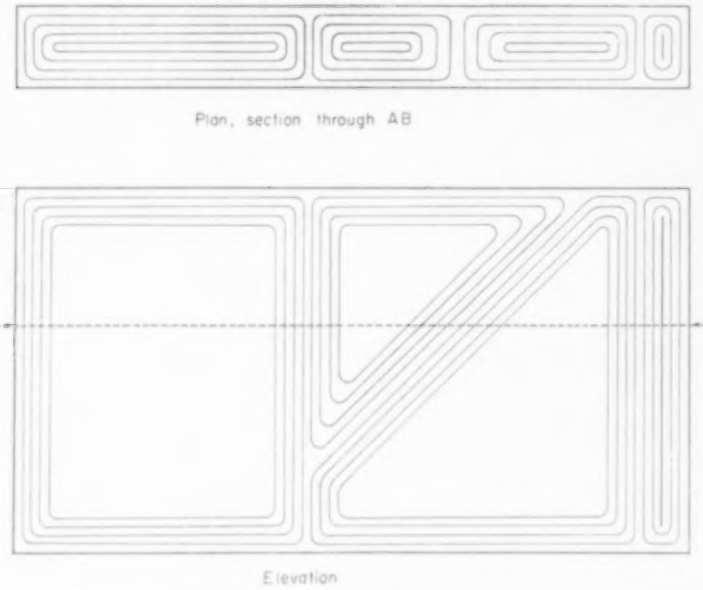

FIG. 6. An arrangement of layers in a cell which would result in the appearance of uniform areas.

clear uniform areas to be layers lying parallel to the cover glass, the whole arrangement suggests groups of boxes, one inside the other, and each such group packed closely among its neighbours (Fig. 6). Actually, as we shall see, it is not necessary to assume the existence of such *layers* as distinct from *planes* in which the preferred orientation of the molecules is uniform.

The uniform areas when seen between crossed polars are generally brightly and uniformly coloured. On rotating the stage of the microscope this colour does not change, but if the analyser is rotated, the colour changes continuously. The colour, in fact, arises from a very high optical rotatory power with a corresponding optical dispersion, so that the extinction position for light of each colour differs considerably and consequently when using white light some colour is always subtracted whatever the position of the analyser. If the concentration was not too high, areas which were sufficiently large and uniform for quantitative observations were frequently obtained, but with higher concentration the experimental difficulties increased. With the specimens which

gave these suitable areas the optical rotation could be measured correct to about 3° by using the polarization microscope as a polarimeter.

The structure in the liquid-crystalline phase

For the purpose of this general account of the phenomenon it will be convenient at this stage to describe the structure which we have put forward to explain these observations, rather than to give the detailed arguments which lead to its proposal and which can be found in the original papers.[6–8] Afterwards we shall consider the quantitative observations which confirm our belief in the existence of this structure.

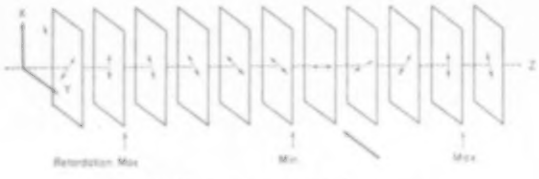

FIG. 7. Model of the twisted structure.

The structure may be looked upon as derived from a nematic structure by superimposing on it an axis of torsion of uniform pitch at right angles to the long axes of the molecules. Consequently if we consider planes at right angles to this axis, the preferred orientation of the molecules, though always at right angles to it, will change continuously along its length and the same orientation will be repeated at equal intervals. The model is shown in Fig. 7 where Z is the axis of torsion and X, Y, Z is a cartesian coordinate system. A number of equally spaced planes at right angles to Z and some arbitrary distance apart are shown . The long axes of the molecules lie in these planes and their orientation in each one is indicated by a double headed arrow. If a volume of solution is observed at right angles to the Z axis, maxima in the major refractive index and birefringence will be observed along parallel lines where the molecules are at right angles to the direction of observation and minima where the molecules are parallel to the direction of observation, so that the periodicity S, will equal half the pitch of the torsion. On the other hand, if the same volume is observed in a direction parallel to the axis of torsion, no periodicities will be seen, but a high degree of optical rotation will be observed. Although S is dependent on solvent, concentration and temperature the *structure* will be as shown in Fig. 6 in any solution in which these are uniform. However, the orientation of the structure may vary from place to place throughout the solution, and the three dimensional pattern thus produced may be called the *texture* of the solution (following the use of this word by Friedel[3] when describing liquid-crystals.) Thus we may have various *textures* but all will have the same *structure*. A special case is that of the spherulite where the nature of the texture arises from the spherical shape imposed by the interfacial tension. A very elegant explanation of the spherulitic pattern has been given by Pryce and Frank[8] and this is consistent with the structure of any small volume of the spherulite (small compared to the spherulitic pattern, but large enough to contain many molecules) being as shown in Fig. 7 except in the vicinity of the line of disinclination. Figs. 10 and 11 of reference 6 show two different textures, each of which has formed in a capillary.

[7] C. Robinson and J. C. Ward, *Nature, Lond.* **180,** 1183 (1957).
[8] C. Robinson, J. C. Ward and R. B. Beevers, *Disc. Faraday Soc.* **25,** 29 (1958).

De Vries' theory of cholesteric liquid crystals

A similar twisted structure has been proposed by de Vries[9] to explain the optical rotatory power of some esters and ethers of cholesterol. These materials show a very high form-optical-rotation which is associated with periodicities, smaller than those we have so far described as occurring in the polypeptides, but which can be made visible as bright and dark lines by filling a wedge-shaped space in cleft mica with the substance. These have been interpreted by some authors, including Friedel, as intersections of planes (Grandjean planes) in the liquid crystal by the boundaries. De Vries derives an equation relating the optical rotatory power to the pitch of the torsion, the wavelength of the light and the birefringence of what may be called the untwisted medium. Data were not available for him to verify this relaionship for cholesteryl compounds, but the polypeptide solutions with their larger spacings, the size of which can be varied over a wide range by altering the concentration or the solvent, are more suitable for the necessary measurements. When the spacings in the polypeptide solutions are large enough, a simplified form of de Vries' relation may be used, as has been shown by Dr. Ward of this laboratory. The conditions under which this simplified equation holds may be explained as follow. (For a full understanding of de Vries' theory his original paper should be consulted).

A major difficulty in developing a theory of the twisted structure (Fig. 7) is that the dielectric "constants" are here a function of Z, the axis of torsion. De Vries deals with this by transforming to new axes ξ, η which twist round with the structure, so that in these axes the dielectric constants do not vary with Z. These are related to the stationary X and Y axes by

$$X = \xi \cos \frac{2\pi Z}{P} - \eta \sin \frac{2\pi Z}{P}$$

$$Y = \xi \sin \frac{2\pi Z}{P} + \eta \cos \frac{2\pi Z}{P}.$$

The formula which de Vries gives for the optical rotation is

$$\Theta \left(= \frac{d\psi}{dZ} \text{ in de Vries} \right) = - \frac{2\pi}{P} \frac{\alpha^2}{8\lambda'^2(1 - \lambda'^2)} \qquad (1)$$

where $\Theta = \dfrac{d\psi}{dZ}$ is the optical rotation of a uniform area per unit thickness, $\alpha = \dfrac{\varepsilon_2 - \varepsilon_1}{2\varepsilon}$ (where ε_1 and ε_2 are the principal dielectric constants along the two axes ξ and η which twist round with the structure, $\varepsilon = \frac{1}{2}(\varepsilon_1 + \varepsilon_2)$, P is the pitch of the helix and $\lambda' = \dfrac{\lambda}{P\sqrt{\varepsilon}}$.

The two principal refractive indices of the untwisted material are then $n_1 = \sqrt{\varepsilon_1}$ and $n_2 = \sqrt{\varepsilon_2}$. Then the birefringence of what here will be called the untwisted material may be denoted by $n = n_2 - n_1$ (where $n_2 > n_1$) and its average refractive index, $\frac{1}{2}(n_1 + n_2)$, by N. In order to compare the theoretical conclusions with our experiments a relation between α and n is required.

$$\alpha = \frac{\varepsilon_2 - \varepsilon_1}{2\varepsilon} = \frac{n_2^2 - n_1^2}{n_2^2 + n_1^2} = \frac{2nN}{2N^2 + n^2/2}$$

[9] H. de Vries, *Acta Cryst.* **4**, 219 (1951).

In practice n is always small compared with N, so that $n^2/2$ may be neglected compared with $2N^2$, so we may write

$$\alpha = \frac{n}{N}$$

and hence de Vries' equation (1) may be written

$$\Theta = -\frac{2\pi n^2 P}{8\lambda^2(1 - \lambda^2/P^2N^2)} \tag{2}$$

When $\frac{\lambda^2}{P^2N^2}$ is small compared with unity the formula

reduces to

$$\Theta = -\frac{\pi n^2 P}{4\lambda^2} \quad \text{radians/micron}$$

or

$$\Theta = \frac{-n^2 P}{\lambda^2} \times 4{\cdot}5 \times 10^4 \text{ degrees/micron} \tag{3}$$

where P and λ are in microns. The negative sign indicates that the optical rotation is in the opposite sense to that of the helical twist of the torsion. This is an important point since it means that the sense of the twist can be determined simply from the sign of the optical rotation. (This formula is identical with that derived by Maugin[10] for a pile of birefringent plates arranged helically by using the Poincaré sphere and rolling cone, to explain the optical rotation which is produced by rotating the cover glass over a nematic liquid crystal (azoxyphenetol) through an angle. His treatment neglects reflections at the interfaces between the plates and assumes that the thickness of the plates are large compared to the wavelength of light. The formula gives results which agree with experimental observations in cases where the pitch is low, but, as will be shown later, the more exact equation derived as in de Vries' theory by using the electro-magnetic theory and assuming a continuous twist is needed to explain the results when the pitch is small).

Verification of the simplified de Vries' equation

Even after considerable experience the formation of satisfactory uniform areas still remained largely a matter of chance, depending no doubt on factors which are difficult to control, such as the condition of the glass surface, and chance impurities which may act as nuclei. It was sometimes necessary to let the cell stand for several weeks before suitable areas appeared. The difficulties increased as the size of the periodicities decreased and it has not yet been found possible to obtain values of Θ corresponding to the lower values of S.

In general Θ was measured for six different wavelengths (λ = 436, 509, 546, 578, 589 and 644 mμ) in each experiment. Since the rotation was often considerably greater than 180°, to obtain the absolute value it was necessary to take readings on several areas of different thickness which had been allowed to form in a wedge-shaped cell (this procedure also showed that the rotation was proportional to the thickness, or very nearly so, which otherwise could not have been assumed). In all the solutions whose optical rotation has so far been measured, which had values of S lying between 5 and

[10] M. C. Maugin, *Bull. Soc. Fr. Min.* **34**, 6, 71 (1911).

100 μ, on plotting Θ against $1/\lambda^2$ a straight line was obtained which cut the abscissa near the origin ($1/\lambda^2 \simeq 0{\cdot}5$, where λ is in μ). When this had been established it was found possible to dispense with the use of a wedge-shaped cell to determine the sign and the absolute value of the rotation. Fig. 8 shows the dependence of Θ upon $1/\lambda^2$ in a typical experiment.

Except in the higher concentrations, where the periodicities become difficult or impossible to resolve, S, that is P/2, can generally be measured without difficulty by direct microscopic observation (with methylene chloride however the structure seemed

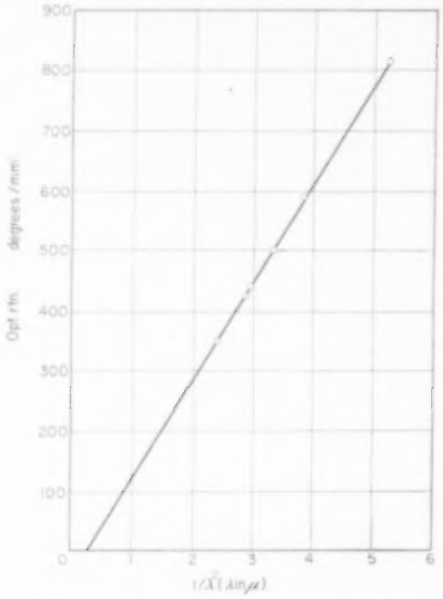

FIG. 8. Dependence of optical rotation on wavelength. PBLG in chloroform. 18 g/100 g.S = 25 μ.

unstable over a considerable range of concentration within which consistent results were not obtained). Fig. 9 shows the dependence of S upon concentration for solutions in dioxan. Here approximately $S = k_1/C^2$ where k_1 is a constant and C is the concentration in ml/100 ml. A similar concentration dependence was found in the other solvents. No significant dependence upon molecular weight was found.

Table 1 shows 15 results on several preparations of PBLG and PBDG covering a range of molecular weights, concentrations and solvents. R9 and R5 were specimens of PBLG in which the molecules contained approximately 670 and 270 residues respectively. D1 was a specimen of PBDG of similar, but undertermined, molecular weight. The addition of 10 per cent ethanol in three of the experiments not only reduced the size of the spacing but reduced the time required to obtain suitable uniform areas. In column 6 are given the values of n, the birefringence of the untwisted structure, calculated from the de Vries equation and divided by the volume fraction. All these results, except for a single result for meta-cresol and one obtained with chloroform and ethanol as solvent, fall within 0·212 and 0·308. (Calculations showed that using the simplified

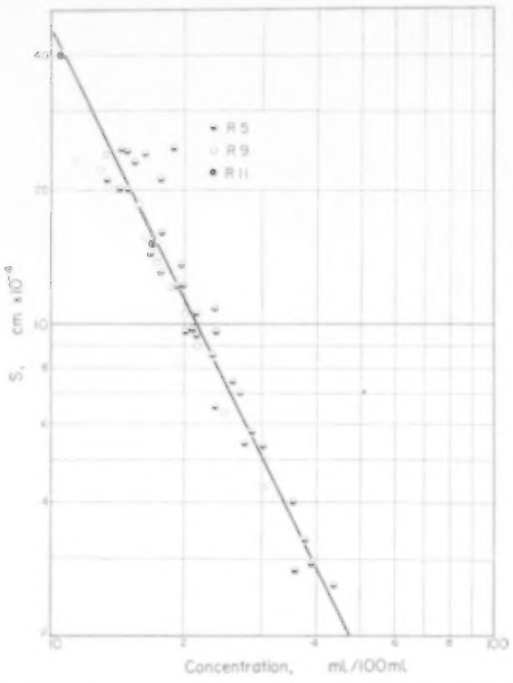

FIG. 9. Double logarithmic plot of S against concentration. PBLG in dioxan.

TABLE 1. BIREFRINGENCE OF THE UNTWISTED MEDIUM AS CALCULATED FROM THE OPTICAL ROTATION AND THE MICROSCOPIC SPACING

Solvent and polymer	Volume fraction	Spacing (μ)	Opt. rot. / mm	Opt. rot/mm / vol. fracn.	Calc. bir. / vol. fracn.
CH_2Cl_2, R9	0·114	100	−157	−1408	212
CH_2Cl_2, R5	0·238	24	−255	−708	277
CH_2Cl_2, *R5	0·184	20	92	383	260
$CHCl_3$ R5	0·276	25	499	1265	308
$CHCl_3$, R5	0·309	18	310	770	259
$CHCl_3$, *R5	0·275	9	92	257	236
$CHCl_3$, *R5	0·350	4·8	74	162	177
Dioxan, R5	0·140	20	59	329	235
Dioxan, R5	0·210	11	56	204	218
Dioxan, R5	0·274	6·5	75	210	250
Dioxan, R5	0·292	5	90	236	266
Dioxan, R9	0·251	6·4	82	251	284
Dioxan, D1	0·211	6·5	−58	−212	246
D.C.A, R9	0·330	11·8	158	370	239
				average	248
m-Cresol, R5	0·242	6·7	90	353	400

* = +10% Ethyl alcohol

rather than the exact de Vries equation would not be expected to introduce an error of more than 4 per cent in any of these cases.)

It remained to obtain an experimental value for n. The model proposed for the structure suggests that it might be possible to obtain this from maxima in the periodic retardation in the twisted structure. In favourable cases values could be obtained in this way, but, because of the small periodicity and other factors the results were very rough. However the values obtained fell within the limits of those calculated in Table I. It should be remembered that without the de Vries relationship there would have been no way of forecasting even the order of magnitude of the optical rotation, so that even these rough values were of interest. A more reliable value, however, was obtained from a racemic mixture of the D and L enantiomorphs (e.g. a solution containing equal quantities of PBLG and PBDG). This forms a birefringent solution which show no periodicities, but properties characteristic of a nematic liquid-crystalline material, including the "threads" from which the name is derived. When such a solution containing 20·8 ml/100 ml of total polymer was introduced into a capillary it became oriented spontaneously and had a birefringence that was uniform throughout the capillary. The birefringence divided by the volume concentration was found to be 0·029 and this value was reproducible. In a flat cell, 1 mm deep, a value of 0·026 was given by discrete areas in the same solution. The birefringence divided by the concentration was then calculated from the values of Θ and S given by a solution of the same concentration containing PBLG only. This value was also 0·026. It seems, therefore, justifiable to assume that the nematic structure observed in the racemic mixture corresponds to the untwisted form of the structure formed in solutions of the optically active polypeptide. The good agreement between the calculated and observed values, therefore, not only show that de Vries equation holds for the structure proposed for PBLG, but demonstrates quantitatively that this may be looked upon as a twisted structure derived from the nematic structure, this twist arising from either the right- or left-handedness of an L or D enantiomorph. How much the scatter of the results in Table1 is due to the value of n being dependent on concentration and solvent and how much due to experimental errors remain, a matter of conjecture. (In some of the experiments the determination of concentration introduced a considerable error.)

The phenomena when S *is small*

So far we have only considered cases where P is large enough to make λ^2/P^2N^2 small compared to unity, so that de Vries' equation may be written in the simplified form of equation (3). However we see from equation (2) that if P is progressively decreased so that λ^2/P^2N^2 passes through unity the optical rotation will change sign. This non-linear relationship and change of sign of the optical rotation in passing through a narrow band of wavelengths is what is found in the liquid crystals of the cholesteryl compounds and which is there associated with the small periodicity of the Grandjean planes. A very striking characteristic of these compounds is their property of reflecting bright iridescent colours when illuminated with white light. The colours are reflected in accordance with the Bragg equation

$$n\lambda = 2\,\mathrm{S}\sin\theta$$

where n is the order of the reflection, λ is the wavelength of the light, S is the distance between the planes at which reflection occurs and θ is the angle between the planes and

the direction of the incident light. Since we are concerned with visible light and not X-rays, λ will here be the wavelength in the *medium*, that is to say the wavelength of the light *in vacuo* divided by the refractive index of the solution. (Compare Oster's treatment of iridescent bodies formed by tobacco mosaic virus.[11]) The value of S obtained from this relationship agrees with that determined by microscopic observation of the Grandjean planes. When $S = \lambda/2$, θ will be 90° and therefore visible light will be reflected normally when S is between approximately 0·4 and 0·2 μ. De Vries' theory of the twisted structure is successful in explaining these properties and also the fact (observed in the cholesteric liquid crystals) that, in the region of reflection, one circularly polarized component of the incident light is totally reflected while the other passes through unchanged and, further, that the rotation of the electric vector of the reflected light is, contrary to what is found in normal substances, in the opposite sense to that of the incident light.

If the twisted structure given by the polypeptide solutions only differs quantitatively from that found in the esters and ethers of cholesterol, one would expect the iridescent colours to be shown by the polypeptide solutions when the value of S was of suitable size. Iridescent colours had in fact been observed with solid films slowly evaporated from a solution of PBLG. Recently however the phenomenon has been found in solutions of poly-γ-ethyl-L-glutamate (PELG) in ethyl acetate where very small spacings can be obtained in solutions which are still sufficiently fluid to allow them to be transferred easily from one vessel to another.

These solutions, if the concentration is suitably adjusted, reflect brilliant colours which depend on the direction of the incident light and of observation. When such a solution is introduced into a capillary tube and is illuminated with a parallel beam of white light a continuous spectrum of colour is observed if the reflected light is allowed to fall on a white surface. If corresponding observations are made with monochromatic light it is found that each wavelength is reflected at the angle which would be expected if it is assumed that the solution contains reflecting planes which are all equally spaced and that groups of these planes are oriented at every angle to the incident light (the experiment in fact being the optical analogue of an X-ray powder diagram.) That the planes in which the molecules lie and have equal orientation are so oriented is to be expected from the microscopic observations on the PBLG solutions of higher periodicities. The nature of these reflections is well illustrated in the following experiment. PELG was dissolved in ethyl acetate to give a concentration of 29·0 g in 100 g of solution. The solution was then somewhat further concentrated until, on illuminating with white light a colour towards the red end of the spectrum was reflected back parallel to the direction of the incident light. The solution was mixed on a very slow stirrer until the reflected colours showed the concentration to be uniform (this takes some days due to the high viscosity, low diffusion coefficient and large dependence of S on the concentration) and was then introduced into a glass tube, 0·5 mm in diameter, which was afterwards sealed at both ends and immersed in a large thermostated beaker of water. When the tube was illuminated at 20° only first order reflections were seen. The angle ϕ which the reflected light made with the incident light was measured for six wavelengths. This could be read correct to $\pm 2°$. On raising the temperature to 30°, the value of ϕ increased considerably for each wavelength and some second order reflections appeared with lower values of ϕ. After making corresponding

[11] G. Oster, *J. Gen. Physiol.* **33**, 445 (1950).

observations at 40° the temperature was again reduced to 20° and the readings at this temperature repeated. θ, the angle which the incident light makes with the set of parallel planes from which reflection takes place, is given by $\theta = 90° - \phi/2$. λ was obtained by dividing λ_v, the wavelength *in vacuo*, by 1·4, the approximate refractive index of the solution. The values of S, calculated from the Bragg equation are given in Table 2. Within the experimental error, which arose chiefly from the crudeness of the optical system, the same value was obtained for each wavelength and also on returning to the original temperature. The value in this and similar sealed tubes was found to be unchanged after several weeks. It will be seen that the value of S nearly doubled on

TABLE 2. S, CALCULATED FROM ϕ AT DIFFERENT TEMPERATURES

	λ_v (in mμ)						Average: all wavelengths
	436	480	509	546	589	644	
ϕ, in degrees at 20°	70	65	40	13	—	—	
ϕ, in degrees at 30°	115	110	95	95	90	70	
ϕ, in degrees at 40°	135	130	120	120	110	105	
ϕ, in degrees at 20°	70	55	20	15	—	—	
S × 10², in μ at 20°	19	19	19	20	—	—	19
S × 10², in μ at 30°	29	30	27	29	30	28	29
S × 10², in μ at 40°	41	41	36	39	37	38	39
S × 10², in μ at 20°	19	19	18	20	—	—	19

increasing the temperature by 20°. (Beevers in this laboratory had previously found by microscopic observation that in a solution of PBLG in dioxan S increased from about 8 to 13 μ between 25° and 45°. With cholesteryl compounds, according to Friedel,[3] the temperature coefficient of S is positive for some and negative for others.)

The optical rotation in the region where these reflected colours are obtained would be expected to show the reversal of sign observed with the cholesterol compounds. This had not up to the present been verified since uniform areas suitable for the observations have not been obtained in these highly viscous solutions. Nor, has it yet been possible to show that the reflected light is circularly polarized in the expected sense, since this requires the planes to be more uniformly oriented.

We may here point out an important difference between the nature of the optical rotation of normal substances and of cholesteric liquid crystals. While the more familiar cases of optical rotation, including that produced by the α-helix, have their origin in the selective *absorption* of one circularly polarized component of the light, the "form"-optical-rotation of the twisted structure in polypeptides and in esters and ethers of cholesterol originates in the selective *reflection* of one circularly-polarized component of the light. In the first class we have the Cotton effect for wavelengths in the neighbourhood of the absorption band, while in the second class iridescent colours are observed in the neighbourhood of the reflection band and there is a change of sign of the optical rotation on crossing this band. Further, while in the first class the wavelengths of the absorption band is determined by the chemical constitution of the material, in the second class the wavelengths of the *reflection* band is entirely determined by the pitch of the torsion in the twisted structure and the birefringence of the untwisted medium.

Since the specific rotation of the twisted structure in the PBLG solutions which were investigated was 20,000° to 140,000°, any contribution directly due to the α-helices could safely be neglected.

Reversal of the sense of the twist

It will be seen from Table 1 that while the form optical rotation given by the PBLG solution in most solvents was positive, in methylene chloride it was negative. This cannot be due to the solvent affecting the sign of the birefringence of the untwisted material, since n appears in the equation as its square. It would seem, therefore, if the equation is valid, that there must be a change in the sense of the twist of the torsion

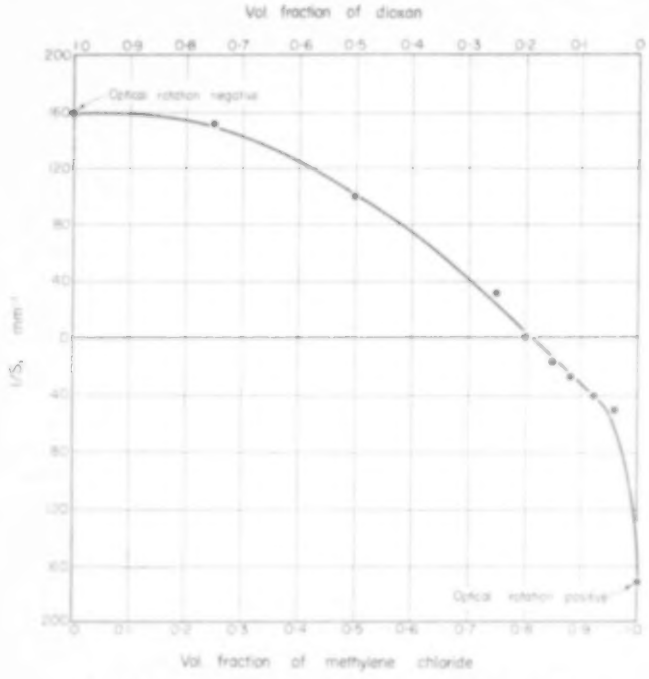

FIG. 10. Dependence of S on solvent composition for mixtures of methylene chloride and dioxan.

in passing from, say, dioxan to methylene chloride. That this is in fact the case was shown by observing solutions in a series of mixtures of these two solvents. Instead of the optical rotation, the more easily measured quantity, S, was determined. The results are shown in Fig. 10 where $\frac{1}{S}$ is plotted against the percentage of dioxan in mixtures of this and methylene chloride. For convenience, the value of S in methylene chloride is plotted as a positive value and that in dioxan as a negative value. It will be seen that the curve cuts the abscissa at 0·8 volume fraction of dioxan at which point S may be considered infinite. Observations in the immediate neighbourhood of this point showing no periodicities but only nematic threads, as would be expected with a completely untwisted structure. The twist therefore, becomes progressively looser as

this point is approached from either side and, after it is reached, the structure again becomes progressively more highly twisted, but in the opposite sense.

These results show clearly that there is no simple relationship between the sense of the twist of the macrostructure and the sense of the α-helix since the latter is the same in both solvents, as is shown by the value of b_0 in the Moffit equation for the optical dispersion being much the same for each solvent.[12]

The molecular arrangement in the structure

The agreement of the results with the de Vries equation and other evidence which has been discussed here and in the original papers shows that we may have some confidence in the model correctly representing the distribution of molecular *orientations* within the structure. It remains to discuss how the *molecules* are distributed and how their distribution varies with concentration.

X-ray photographs of the birefringent phase showed a ring indicating a spacing, d, which increased continuously with concentration from 18 to 29 Å over a concentration range of 16·6 to 48·5 ml/100 ml. As the concentration increased this ring became less diffuse. If the preparations became oriented in the process of filling the capillaries which were used for the x-ray photographs, two more or less pronounced equatorial spots or arcs appeared on the ring. In no case, with the exposures used, were any other reflections observed which varied with concentration. In the liquid crystalline structure formed by tobacco mosaic virus (TMV) Bernal and Fankuchen[13] obtained in the more concentrated systems (their dried gels) four equatorial reflections corresponding to the spacings d, $d\sqrt{3}$, $d\sqrt{4}$, $d\sqrt{7}$, which is consistent with the long shaped particles forming a two-dimensional hexagonal array. On dilution the spacings increased in size but the relationship was maintained as long as the diminished intensity of the lower reflections allows them to be observed; thus in the *solutions* seldom more than the first two reflections were seen. Although only one such relection was observed in the PBLG, it still seemed reasonable to expect that the structure might be a similar two dimensional hexagonal array, with the addition of an axis of torsion at right angles to the long axes of the molecules (the higher reflections not being observed probably because of the greater fluidity of the system). The amount of torsion per molecule required to give the observed values of S is very small, so that its effect on the structure of a small region which is nevertheless large enough to give a sharp X-ray reflection, would be expected to be negligible.

The theoretical value of d for the hexagonal array may be calculated from the equation

$$C = \frac{W\sqrt{3} \times 10^{24}}{2Nd^2q\rho} = \frac{1{\cdot}6 \times 10^4}{d^2}$$

where C is the concentration in ml/100 ml; W = 219, the residual weight; N = Avogadro's number; d is the distance between the $(10\bar{1}0)$ planes in Å; q = 1·5 Å, the length of the projection of one residue on the axis of the α-helix; and ρ = 1·3, the assumed density of the polymer. In deriving this equation, dilution in a direction parallel to the long axes of the molecule has been neglected; such dilution will however be small owing to the high axial ratio of the molecules. In Fig. 11 the experimental

[12] A. R. Downie, private communication.
[13] J. D. Bernal and I. Fankuchen, *J. Gen. Physiol.* **25**, 111 (1941).

values are shown in a double logarithmic plot and the theoretical curve obtained from this equation is shown as a broken line. The agreement between the calculated and experimental values suggests that the structure consists of parallel rods having, in a plane at right angles to their length, something between a two dimensional hexagonal arrangement and a random arrangement, the arcs in the X-ray photographs showing, however, that the arrangement is far from random. Further the absolute value of d obtained shows beyond doubt that the structure is composed of discrete single molecules and not of complexes consisting of two or more molecules.

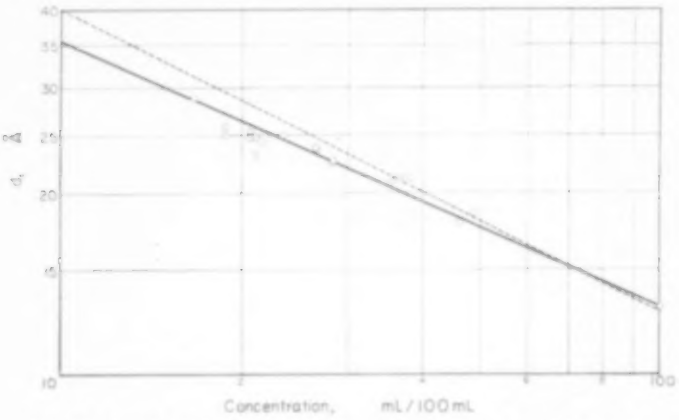

FIG. 11. Double logarithmic plot of d against concentration PBLG in dioxan.

It follows from these considerations that if we want to calculate the angle of twist per molecule in the Z direction and the distance between the molecules at different concentrations it will be instructive to base these calculations on the assumption of a two dimensional hexagonal array. The number of molecules which the Z axis passes through in a distance equal to the periodicity will be given by S/d and in the more dilute solutions of, for instance, PBLG in dioxan, this can be as high as 10,000. Since θ, the angle per molecule in the Z direction, is equal to $d/S \times 180^\circ$, it will in this case be of the order of 1 minute. Since for the concentration of dioxan solutions which have been investigated $S \propto \frac{1}{C^2}$ and $d \propto \frac{1}{C^{1/2}}$, we see that $\theta \propto C^{3/2}$. So we find that the angle of deviation from the parallel position is not only very small even in the higher concentrations, but decreases on dilution. The polypeptide solutions which have been found to give the twisted structure all have their molecules in the α-helical configuration. Each molecule presents a helical arrangement of dipoles and, since all the helices in a solution are of the same sense, we may expect the intermoleuclar attractions arising out of the dipoles to impose a uniderectional twist on the array of parallel molecules.* The smallness of the angle and the fact that it decreases continuously on dilution suggests that it arises from a dynamic equilibrium, but it is at present not easy to see what factors determine its value.

* The sense of the twist could conceivably be either the same as, or opposite to, the sense of that the α-helices from which the twisted structure is built up, since although in a left-handed helix the 1st, 2nd, 3rd, 4th, etc. residues form a left-handed helix, if we consider the 1st, 4th, 9th, 13th, etc. residues, they form a right-handed helix. This may partly explain the experimental observation that changing the solvent may alter the sense of the twist without altering the sense of the α-helix.

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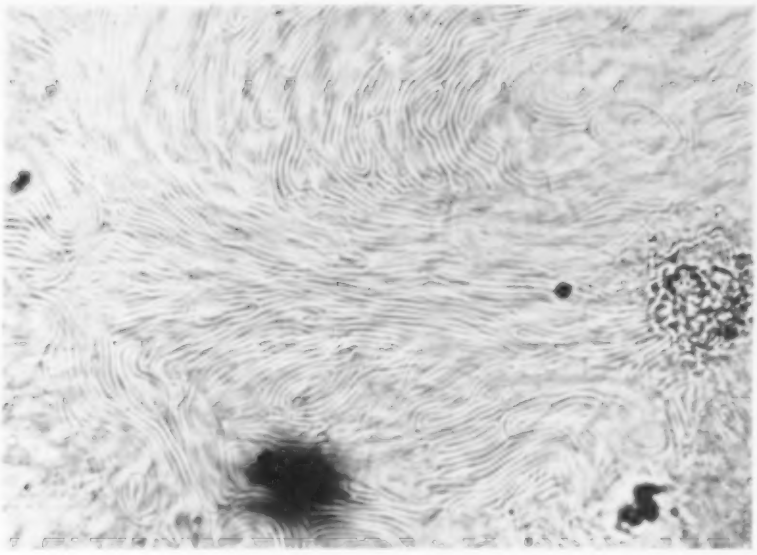

FIG. 12. Periodic lines in solution of DNA in 0·1 M NaCl. S $\simeq$ 1 μ. Cell thickness 0·1 mm. Natural light.

FIG. 13. Periodic lines and uniform areas in solution of DNA in 0·1 M NaCl. Cell thickness 0·01 mm. Natural light.

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From models and the known dimensions of the α-helix it is found that the diameter of the cylinder described by the PBLG molecule with side-chains fully extended when rotated about its long axis is 28·2 Å. By subtracting this value from the intermolecular distance, $2D/\sqrt{3}$ we obtaine D, the distance of nearest approach of two side-chains in neighbouring parallel molecules at a given concentration.[8] This value of D for any given molecular weight will be greatest at the B point where the birefringent phase is at its lowest concentration. In the molecular weight range studied, it varied from 14·4 to −3·8 Å, the negative values showing that the side chains could overlap, in which case very slight movements of the side chain would bring adjacent parallel molecules into contact. But even where D has a positive value, since the length of the α-helix is always many times greater than D, the angle which its long axes must rotate through in order to make contact with a neighbouring molecule will always be very small (less than 1·5° in the cases investigated).[8] Very slight oscillations will consequently produce periodic contacts. It therefore does not seem necessary to assume that long range forces play a part in the formation of the structure, but, on the other hand, one can easily imagine how periodic contacts between the molecules in the model of Fig. 7 would result in a structure having the nature of a delicate three-dimensional network. Such a network would account for the fact that if a polypeptide solution containing visible periodicities is observed under the microscope, it is seen that the visible pattern shows elastic recovery under very slight shearing forces and that a slight but definite yield value must be exceeded before irreversible flow takes place.

The twisted structure in other systems

Qualitatively similar structures to those which have been described in detail for PBLG have been found in solutions of several other polypeptides in organic solvents, including PELG, PEDG and poly-γ-methyl-L-glutamate. In all these solutions the polypeptide molecules are known to form the α-helix configuration. This is evidently so even for solutions of PBLG in dichloracetic acid, for although Yang and Doty[14] found the random coil configuration in comparatively dilute isotropic solutions in this solvent, Downie *et al.*[15] found from the optical rotation that on heating a birefringent solution until it became isotropic that it was evidently in the α-helical configuration.

It has been shown[16,17] that poly-β-benzyl-L-aspartate forms an α-helix of the opposite sense to that of poly-γ-benzyl-L-glutamate. It was therefore of interest to find that a solution of a copolymer of equal proportions of β-benzyl-L-aspartic acid and γ-benzyl D-glutamic acid in chloroform, formed the twisted structure.

It seems probable that the rigidity of the molecule when in the α-helix configuration is an important factor in the formation of the birefringent phase, while the arrangement of the dipoles in a left- or right-handed helix gives rise to the twist characteristic of the structure. Robinson *et al.*[8] suggested that it might be possible to find in biological systems similar twisted structures which had previously been overlooked. Since then, Robinson (unpublished) has examined concentrated aqueous solutions of deoxyribonucleic acid [DNA] where again we have long-shaped, optically active, helical molecules. A solution containing about 6 per cent of DNA dissolved in 0·1 M NaCl

[14] J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957).
[15] A. R. Downie, A. Elliot, W. E. Hanby and B. R. Malcolm, *Proc. Roy. Soc.* A **242**, 325 (1957).
[16] A. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).
[17] E. M. Bradbury, A. R. Downie, A. Elliott and W. E. Hanby, *Nature, Lond.* **187**, 321 (1960); *Proc. Roy. Soc.* A **259**, 110 (1960).

(the solution was diluted under microscopic conditions, and the concentration was only very roughly estimated) when introduced into a cell 0·1 mm deep showed periodic lines after standing for several days. These lines (Fig. 12), whether observed in natural light or between crossed polars, resembled those in the polypeptide solutions. The value of S was in the neighbourhood of 1μ but increased appreciable on dilution. The concentration range over which these structures were observed was, however, small compared to that for the polypeptide solutions. So far it has not been possible to obtain uniform areas in a cell of this thickness, but in a 0·01 mm cell, areas which contained no lines but which were surrounded by periodic lines appeared (Fig. 13). Although the general appearance of these areas suggested that they were the uniform areas of the twisted structure, they were almost black between cross polars and the optical rotation, if any, was too small to measure, which would, however, be expected in a cell of this thickness unless the rotation was more than about 200° or 300° per millimetre of solution.

These experiments with DNA were of a preliminary nature and it is possible that the material was partly denatured when the structures described were observed. It seems nevertheless very probable that we were observing another example of the twisted structure, although this well not be proved until measurements of the optical rotatory power are obtained. It would however be of considerable interest if the existence of the twisted phase in biological systems could be substantiated. It is tempting to think that this highly organized liquid-crystalline phase, which can dissolve other components without its qualitative nature being changed, may be found to play a part in providing orientation in chemical reactions involving some of the highly specific, optically active molecules found in nature.

Acknowledgements—My thanks are due to Dr. J. C. Ward for his interpretation of the de Vries theory in a way that allowed it to be applied to the observations of the polypeptide solutions. Without his treatment of the theory the present paper would not have been written.

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A MANY ELECTRON MODEL FOR OPTICAL ROTATION

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THE quantum mechanical expression for optical rotation of a molecular system was obtained by Rosenfeld.[1] A convenient form of this equation is given by Eyring *et al.*[2] where φ is the optical rotation in radians per centimeter.

$$\varphi = \frac{16\pi^2}{3ch} N_1 \left(\frac{n^2+2}{3}\right) \sum_a \rho_a \sum_b \frac{\nu^2 R_{ba}}{\nu_{ba}^2 - \nu^2} \tag{1}$$

Here R_{ba} is the imaginary part of the dot product of the matrix elements for the electric and magnetic moments.

$$R_{ba} = \mathrm{Im}\left\{(a\,|e\mathbf{r}|\,b)\cdot(b\,|\,\frac{e}{2mc}\,\mathbf{r}\times\mathbf{p}\,|\,a)\right\} \tag{2}$$

N_1 is the number of active molecules per unit volume, ρ_a the probability that a molecule is in state a, n the index of refraction, ν_{ba} the frequency of the transition from state a to b, and ν the frequency of the incident light. Using this equation and knowing the complete set of eigenfunctions for the molecule one can calculate the optical rotation. Unfortunately the complete set of eigenfunctions are not known for molecules in general so that a straightforward calculation is impossible. We will discuss a model which exhibits the necessary conditions for optical rotation and show how these conditions are fulfilled in many optically active molecules.

The simplest model capable of exhibiting optical rotation was shown by Condon *et al.*[3] to be an anisotropic harmonic oscillator with a perturbing term of the form $Axyz$. They used the potential:

$$V = \tfrac{1}{2}K_1x^2 + \tfrac{1}{2}K_2y^2 + \tfrac{1}{2}K_3z^2 + Axyz \tag{3}$$

Treating the $Axyz$ as a perturbation they obtained for the optical rotation considering transitions from the ground state

$$\varphi = \frac{16\pi^3 N_1}{\lambda^2}\left(\frac{n^2+2}{3}\right)\frac{Ahe^2}{12(2\pi)^6\mu^3}\left(\frac{1}{\nu_2}-\frac{1}{\nu_3}\right)\frac{1}{(\nu_2+\nu_3)^2-\nu_1^2} \tag{4}$$

$$\left[-\frac{1}{\nu_1^2-\nu^2}+\frac{1}{(\nu_2+\nu_3)^2-\nu^2}\right] + 2 \text{ terms in cyclic permutation of indices 1, 2, 3.}$$

In this equation μ represents the reduced mass of the oscillator and $\nu_1 = \frac{1}{2}\sqrt{\frac{K_1}{\mu}}$ etc. are the frequencies of the ground state transitions. The general expression for the

[1] L. Rosenfield, *Z. Physik.* **52**, 161 (1928).
[2] H. Eyring, J. Walter and G. E. Kimball, *Quantum Chemistry* p. 345. John Wiley, New York (1944).
[3] E. U. Condon, W. Altar and H. Eyring, *J. Chem. Phys.* **5**, 753 (1937).

rotation of a system in the initial state with quantum numbers (n_1, n_2, n_3) is given by Condon *et al.* It is interesting to note that, using the correspondence principle in the limit of large quantum numbers, Planck's constant disappears. Thus, the model shows optical activity in the classical limit. This model is for one electron moving in an electrostatically asymmetric field. Calculations using this model in the sense of an anisotropic harmonic oscillator acted upon by a permanent dipole usually lead to small values of the optical rotation.

We can see from equation (4) that the optical rotation vanishes if any two of the three principal frequencies for the unperturbed oscillator are the same. An expression similar to equation (4) except for the numerical coefficient is obtained for the particle in a box calculation, with the condition that the box has a different length in each of the three principal directions. The form of the expression for optical rotation is dependent on the anisotropy and not on the particular set of eigenfunctions used. The rotation is directly proportional to the perturbing term A. The term in A provides the necessary dissymmetry such that the oscillator has neither a plane nor center of symmetry. The essential features of this model may be characterized by two conditions: first, that $K_1 \neq K_2 \neq K_3$ this is the condition for anisotropy; second, that $Axyz \neq 0$, this is the condition for dissymmetry. Both conditions are necessary for a molecule to show optical rotation.

We now discuss the extension of this one electron model to many electrons.

The transition in the chromophoric group not only changes the eigenfunction of the electron undergoing the transition, which is the one electron effect, but also alters the eigenfunctions of all other electrons as they become polarized in the positive field due to the vacant orbital left by the excited electron. The total change can be described in terms of the resultant positive hole. It is this total disturbance in the molecule which we now want to consider. In order to use equations (3) and (4) to explain one or many electron optical rotation it must be realized that the actual potential includes terms not included in the potential of equation (3). However, equation (3) contains the important terms having the proper anisotropy and dissymmetry to contribute to the optical rotation. A and the K's should be chosen to represent the fictitious one electron potential which would be required to describe the effect of the motion of all the electrons in the transition. The many electron parameter A will be made up of contributions from the various groups perturbing the chromophoric center.

We make the assumption that the transition frequency in one of the principal directions is much smaller than the frequencies in the other two directions. This assumption is justified by the fact that most rotary dispersion data can be expressed as a sum of Drude terms. The most important terms are usually those nearest the visible. We shall choose our right-handed molecular axis so that $K_1 > K_2 > K_3$ and therefore $\nu_1 > \nu_2 > \nu_3$. In this case for the molecular rotation we have

$$\varphi = \frac{16\pi^3 N_1}{\lambda^2}\left(\frac{n^2+2}{3}\right)\frac{Ahe^2}{12(2\pi)^6\mu^3}\left(\frac{1}{\nu_2}-\frac{1}{\nu_3}\right)\frac{1}{(\nu_2+\nu_3)^2-\nu_3^2}\,\frac{1}{\nu_2^2-\nu^2} \tag{5}$$

To apply this model to molecules we choose the Z axis along the most polarizable direction in the chromophoric group. This requires that the axis will, in general, lie along the bond joining the chromophoric group to the asymmetric center. The Y axis will be orthogonal and will lie along the next most polarizable direction and X will be chosen orthogonal to Z and Y such that XYZ forms a right-handed coordinate

system. A chromophoric group will frequently exhibit cylindrical symmetry around the Z axis, in first approximation. The distortion of this symmetry by neighboring groups will fix the direction of the Y axis and the magnitude of this distortion will appear directly in the expression for the optical rotation as the difference $\frac{1}{\nu_2} - \frac{1}{\nu_1}$. The magnitude of $\frac{1}{\nu_2} - \frac{1}{\nu_1}$ will, for example, be large in the case of a carboxyl group which is hydrogen bonded. In the case of a freely-rotating carboxyl $\frac{1}{\nu_2} - \frac{1}{\nu_1}$ will still be different from zero because of the directional polarization of neighboring groups.

Note added in proof: For many chromophores the transition nearest the visible contributing to the optical activity is not a dipole transition in the Z direction as implied in equation (5) but is a magnetic transition which can be described as a displacement of charge distribution from predominantly in the Y direction to the X direction, when the XYZ directions are chosen as above. This magnetic transition will correspond to the harmonic oscillator transition (0,1,0) → (1,0,0) with the frequency $\nu_1 - \nu_2$. The condition on the frequencies can now be written as $\nu_1 > \nu_2 > \nu_3 > \nu_1 - \nu_2$. The contribution to the rotation from this band will then be

$$\varphi = \frac{16\pi^3 N_1}{\lambda^2}\left(\frac{n^2+2}{3}\right)\frac{Ahe^2}{12(2\pi)^6\mu^3}\cdot$$

$$\left(\frac{1}{\nu_1}+\frac{1}{\nu_2}\right)\frac{1}{\nu_3{}^2-(\nu_1-\nu_2)^2}\left[\frac{1}{(\nu_1-\nu_2)^2-\nu^2}\right]$$

It can be seen the contribution to the rotation in the visible still has the same sign as the sign of the parameter A. In the examples given the transitions in the carbonyl and ketone oxygen atoms are a magnetic transition rather than a dipole transition; however, the discussion given of these examples remains correct.

To determine the sign of the contribution to A of a particular vicinal group we note that any group will have its electrons flow into the positive hole left by the excitation of the chromophoric electron to an extent which is proportional to the group's polarizability. This requires that the term $Axyz$ for a group is negative at the position of the group and further that the contribution to A is proportional to group polarizability. We see that the groups which lie in the planes defined by the principal axes of the potential do not contribute to the magnitude of A.

By an analysis of each chromophoric group in a molecule of known configuration we can determine the sign of its contribution to the rotation or, more specifically, the sign of the groups Cotton effect. Of more interest we can determine what the stereochemical environment of a chromophoric group must be to give the observed Cotton effect. Our method yields a rapid determination of absolute configuration in the cases where the stereo-relationship of a compound to known compounds cannot readily be established. This method can also be used to determine the effects of temperature and solvent on molecular configurations. Calculations of optical rotation to determine absolute configuration were carried out by Condon *et al.*, Kuhn[4] and Kirkwood[5].

[4] W. Kuhn, *Naturwiss.* **19**, 289 (1938).
[5] J. G. Kirkwood, *J. Chem. Phys.* **5**, 479 (1937).

After a correction of sign in the latter work all arrived at the absolute configuration assumed in the Fisher convention. Boys[6] who came to the contrary result used a model which is difficult to relate to molecular structure. The correctness of the Fisher convention was further supported by the X-ray determination by Bijvoet[9] of the absolute configuration of sodium rubidium tartrate dihydrate. By normal X-ray methods one can only determine the distances between atoms in a molecule. Since the distances are the same for a molecule and its mirror image, this X-ray method cannot give the absolute configuration. By using X-rays with a frequency on the absorption edge of one of the atoms, in this case the rubidium atom, a phase lag is introduced in the anomalous dispersion. This phase lag makes it possible to tell not only the distances between atoms but in what sequence the various atoms occur, which is all that is necessary for the determination of absolute configuration.

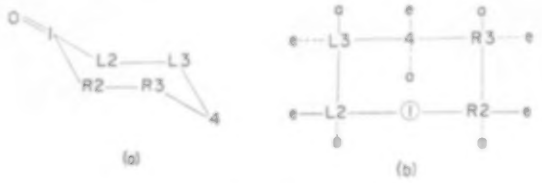

FIG. 1.

As an example of the application of the many electron model we can consider a substituted cyclohexanone. These compounds have been discussed extensively by Djerassi[7] and we will use his notation, calling the substituents a or e depending on whether they are in the axial or equatorial position. Fig. 1b shows the molecule as it would appear looking along the carbon–oxygen bond of the ketone group. Rotary dispersion shows that the ketone is the principal chromophore so that we will choose the potential centering in the carbon–oxygen bond of the ketone. In Fig. 1b we will choose our coordinate system such that Z, the most polarizable direction, is positive into the ring along the C—O bond, Y, the next most polarizable direction, will lie in the plane defined by the atoms $C_1C_{R2}C_{L2}$, in the direction C_{R2}. X will then point toward the top of the page.

In Table 1 we have listed the various substituents and the signs of X, Y, and Z for the octants in which they lie. The substituents will contribute to the A term with a sign

TABLE 1

Position	Substituent	x	y	z	Sign of contribution to A
R2	a	−	+	+	+
	e	0	+	+	0
R3	a & e	+	+	+	−
4	a & e	+	0	+	0
L3	a & e	+	−	+	+
L2	a	−	−	+	−
	e	0	−	+	0

[6] S. F. Boys, *Proc. Roy. Soc.* A **144**, 655, 675 (1934).
[7] C. Djerassi, *Optical Rotary Dispersion* p. 178. McGraw-Hill, New York (1960).

such that the product $Axyz$ is negative and in an amount proportional to their polarizability. This result agrees with Djerassi as to the sign of the contribution to the optical rotation from the groups. The substituents which lie in the planes defined by pairs of principal axes, such as the equatorial substituents on R2 and L2 and the substituents on 4, do not contribute to the sign of the Cotton effect since they do not contribute to A. These substitutents will, however, change the magnitude of the Cotton effect through the terms $\frac{1}{r_2} - \frac{1}{r_1}$.

For lactic acid the chromophoric group is the carboxyl. If the oxygen of the carboxyl is hydrogen bonded to the hydroxide as shown in Fig. 2, the most polarizable

FIG. 2. D-Lactic acid

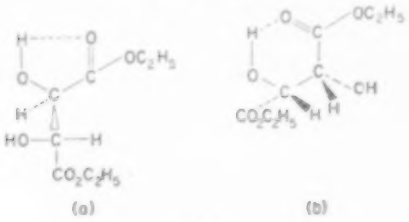

FIG. 3.

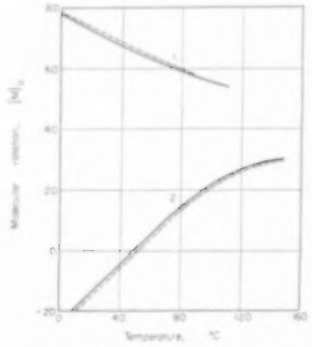

FIG. 4. Molecular rotation of diethyl tartrate in (1) alpha-nitro naphthalene: and in (2) anti-benzaldoxime as a function of temperature.

axis, z, lies along the C=O bond directed toward the carbon; Y lies in the plane of the ring as indicated in the figure with the positive x axis directed upward. The most polarizable group out of the plane is the CH_3 group which lies in the $+x$, $+y$, $+z$ octant. Since the product of $Axyz$ must be negative at the CH_3 position, A will be negative and the rotation will be negative as it is for D-lactic acid.

The solvent and temperature effects on diethyl D-tartrate may be explained by assuming an equilibrium between the intramolecularly hydrogen bonded forms shown in Figs. 3a and 3b and the non-hydrogen bonded freely rotating form.

Fig. 3a shows a five-membered ring structure. We can choose the coordinates on the carboxyl group as in lactic acid and the principal group out of the ring, the $CHOHCO_2C_2H_5$, will be in the $+x$, $+y$, $+z$ direction and thus the rotation will be negative for structure 3a.

Fig. 3b shows an intramolecularly-bonded six-membered ring structure. If we choose the coordinates in the carboxyl as before we have the principal group out of the plane of the ring, the hydroxyl, lying in the $-x$, $+y$, $+z$ octant and A and therefore the Cotton effect will be positive in this case . This will also be the sign of the rotation for the freely-rotating form since the polarization which determines the Y axis is induced by the same large group which tended to orient it into a six-membered ring. The observed data for solvent and temperature effects on diethyl tartrate are shown in Kauzmann and Eyring[8], Fig. 7. The general features of the data excluding the refractive index correction and the change of the rotation of each of the individual forms with temperature, can be expressed by taking the total rotation to be a sum of contributions from the various structures.

$$[M] = [M_d]N_d + [M_1]N_1 + [M_f]N_f$$

Where $[M_d]$ is the molecular rotation of the dextro-rotatory six-membered ring structure, $[M_1]$ and $[M_f]$ are the molecular rotations of the laevo-rotating five-membered ring and of the non-hydrogen bonded form respectively. N_i is the mole fraction of the ith species. Expressing the equilibrium between the forms by the relations

$$N_d = N_f \exp\left(\frac{\Delta H}{RT} - \frac{\Delta S}{R}\right); \qquad N_1 = N_f \exp\left(\frac{\Delta H}{RT} - \frac{\Delta S}{R}\right).$$

The observed values can be represented by choosing suitable values for ΔH and ΔS. Fig. 4 shows two of the experimental curves, shown dotted, and some of the calculated curves, shown with solid lines. The curves 1 are for the alpha-nitro napthalene solvent and were calculated using $\Delta H = 3$ kcal; $\Delta S = 9$ e.u.; $M_d = 100°$ the curves 2 are for anti-benzaldoxime as solvent and the values $\Delta H = 5{\cdot}7$ kcal; $\Delta S = 18$ e.u.; $M_1 = -40°$ were used.

In the case of secondary butyl alcohol the z axis is chosen along the bond between the oxygen and the asymmetric carbon atom, the y axis is chosen normal to this in the direction of the ethyl group, since this is the most polarizable substituent, and x will be orthogonal to z and y. In this coordinate system the principal contribution to A will be from the larger polarizability of the methyl group compared with that of a hydrogen as these are the only two groups not in the planes defined by the principal axes. For secondary butyl derivatives the value of A will be similar, however the optical rotation can vary due to changes in the term $\frac{1}{\nu_2} - \frac{1}{\nu_1}$ as this will be proportional to the polarizability of the chromophoric group and will be larger in the case of secondary butyl iodide than in the case of the alcohol due to the larger polarizability of the iodide.

It should be clear from our examples that this many electron model for optical rotation provides a well founded, general method for predicting the relation between absolute configuration and conformation and the sign of the observed Cotton effects.

[8] W. Kauzmann and H. Eyring, *J. Chem. Phys.* **9**, 41 (1941).
[9] J. M. Bijvoet, *Endeavour* **14**, 71 (1955).

Tetrahedron, 1961, Vol. 13, pp. 241 to 246 Pergamon Press, Inc. Printed in Northern Ireland

REACTIONS OF t-BUTYL PERESTERS—III

CUPROUS BROMIDE-CATALYZED REACTION OF PERESTERS WITH ETHERS

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Abstract—Aliphatic and cyclic ethers react smoothly with t-butyl peracetate and t-butyl perbenzoate at 67–90° in the presence of cuprous bromide. The first products of this reaction are the acyloxy derivatives of the ethers, which are unstable under the chosen experimental conditions and decompose to the corresponding carboxylic acids and unsaturated ethers. Thus, 2,3-dihydrofuran was prepared from 2-benzoyloxytetrahydrofuran in a 66% yield. The unsaturated ethers add t-butyl alcohol to give the corresponding acetals, this step being catalyzed by the carboxylic acid. This reaction is general for ethers containing two adjacent methylene groups, such as n-propyl and n-butyl ethers, tetrahydrofuran, and tetrahydropyran. Diethers, such as 1,4-dioxane and 1,2-dimethoxyethane, give more stable benzoyloxy derivatives, which decompose slowly on prolonged heating to give the expected acetals in low yield.

IN the preceding publications[1,2] the reaction of t-butyl peresters with olefins in the presence of copper salt catalysts was described. It was shown that copper salt catalysts exert a pronounced influence upon the rate of the reaction and the nature of the products. In a subsequent paper[3] the scope of this reaction was investigated. Among other substrates, allyl phenyl ether and 1,4-dioxane were investigated; with the former, t-butyl perbenzoate gave a 50% yield of 3-benzoyloxy-3-phenoxy-1-propene and with the latter a 56% yield of dioxanyl benzoate. In all these reactions the activated hydrogen atom was substituted by the acyloxy group and the t-butoxy group was converted to t-butyl alcohol.

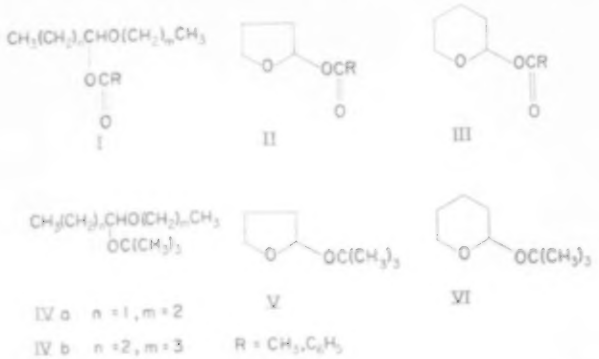
These studies were extended to a number of aliphatic and cyclic mono- and difunctional ethers and to thioethers. The preliminary results of these investigations were reported in recent communications.[4,5] Recently a similar study was initiated by Lawesson *et al.* in Sweden.[6] The details of the reactions of t-butyl peresters with ethers are reported in this paper.

t-Butyl peracetate and t-butyl perbenzoate react smoothly with n-propyl and n-butyl ethers, tetrahydrofuran, and tetrahydropyran in the presence of catalytic amounts of cuprous bromide. In addition to the expected acyloxy compounds, I, II and III, these reactions yielded mainly 1-t-butoxy-1-n-propoxypropane (IVa), 1-n-butoxy-1-t-butoxybutane (IVb), 2-t-butoxy-tetrahydrofuran (V), and 2-t-butoxytetrahydropyran (VI), respectively. The reactions were carried out for 14–48 hr at 67–90°.

[1] M. S. Kharasch and G. Sosnovsky, *J. Amer. Chem. Soc.* **80**, 756 (1958).
[2] M. S. Kharasch, G. Sosnovsky and N. C. Yang, *J. Amer. Chem. Soc.* **81**, 5819 (1959).
[3] G. Sosnovsky and N. C. Yang, *J. Org. Chem.* **25**, 899 (1960).
[4] G. Sosnovsky, *J. Org. Chem.* **25**, 874 (1960).
[5] G. Sosnovsky, Abstracts of Papers presented at the 138th Meeting of the American Chemical Society, New York, Sept. 1960, p. 78P.
[6] S. O. Lawesson, C. Berglund and S. Gronwall, *Acta Chem. Scand.* **14**, 944 (1960).

When the reactions were stopped immediately after all the perester was consumed (4–6 hr), it was possible to isolate substantial quantities of the acyloxy derivatives. Due to their thermal instability, these derivatives could not be purified by distillation sufficiently for elemental analysis. Nevertheless, infrared analysis and the fact that their thermal decomposition led to the formation of the corresponding acid and an unsaturated ether left no doubt that these compounds were the acyloxy intermediates.

These acyloxy derivatives decomposed slowly at 80–100°. At slightly elevated



temperatures decomposition was much faster. Thus, 2-benzoyloxytetrahydrofuran (II) gave a 66 per cent yield of 2,3-dihydrofuran and a 69 per cent yield of benzoic acid after 1 hr at 130°. This method constitutes a new and probably the most convenient route for the preparation of the 2,3 isomer of dihydrofuran.[7,8]

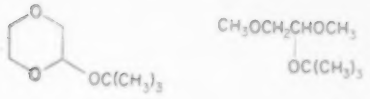
When an acyloxy compound was decomposed in the presence of an equimolar quantity of t-butyl alcohol, the corresponding acetal and carboxylic acid were obtained instead of the unsaturated ether. For example, 2-benzoyloxytetrahydropyran (III) gave 2-t-butoxytetrahydropyran (VI) plus benzoic acid after 14 hr at 80° in boiling t-butyl alcohol. VI was identical in all respects with a sample prepared by the method of Paul[9,10] from dihydropyran and t-butyl alcohol in the presence of concentrated hydrochloric acid.

Dihydropyran reacts with acetic acid at 100° to give 2-acetoxytetrahydropyran, which is reported to be in equilibrium with the starting materials.[11] In the current work it was found that under similar experimental conditions a mixture of equimolar quantities of dihydropyran, t-butyl alcohol, and either benzoic or acetic acid produced mainly 2-t-butoxytetrahydropyran (VI) and only a small amount (13 per cent) of the 2-acyloxy derivative (III). There was only a negligible reaction between dihydropyran and t-butyl alcohol in the presence of cuprous bromide when the carboxylic acid was excluded from the system.

Diethers, such as 1,4-dioxane and 1,2-dimethoxyethane, reacted smoothly with t-butyl perbenzoate in the presence of cuprous bromide to give the expected dioxanyl-benzoate (VII)[5,6] and 1-benzoyloxy-1,2-dimethoxyethane (VIII), respectively. Surprisingly, these benzoates were more stable to heat than those of the monofunctional

[7] D. A. Barr and J. B. Rose, *J. Chem. Soc.* 3766 (1954).
[8] H. Normant, *C. R. Acad. Sci., Paris* **228**, 102 (1949).
[9] R. Paul, *Bull. Soc. Chem.* (5) **1**, 973 (1934).
[10] G. F. Woods and D. N. Kramer, *J. Amer. Chem. Soc.* **69**, 2246 (1947).
[11] J. G. M. Brenner and D. G. Jones, Brit. Pat. 606,764 (1948); *Chem. Abstr.* **43**, 1442 (1949).

VII VIII

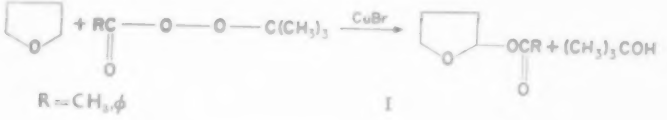


IX X

ethers. Prolonged heating was required for the elimination of benzoic acid, and the yields of the corresponding acetals, (IX) and (X), respectively, were low. Thus, 30 per cent of dioxanylbenzoate (VII) was recovered after it was heated in t-butyl alcohol at 105–110° for 114 hr and 19 per cent of 2-t-butoxy-1,4-dioxane (IX) was obtained. Similarly, VIII gave a 7·5 per cent yield of 1-t-butoxy-1,2-dimethoxyethane (X).

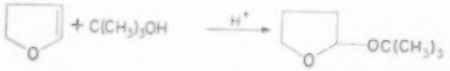
On the basis of these results, a mechanism is proposed to account for the formation of IV, V, VI, IX and X:

t-Butyl peresters react with ethers in the presence of cuprous bromide to form the acyloxy intermediates, which decompose under the chosen experimental conditions to the corresponding organic acids and unsaturated ethers. In the presence of the carboxylic acid, the ether adds t-butyl alcohol to give the observed acetals (IV, V, VI, IX, X).



R = CH_3, ϕ I (1)

V (2)



(3)

Step 1 is assumed to proceed by a concerted mechanism,[2,12,13] and it is generally accepted that esters pyrolyze to olefins by a concerted mechanism also.[14,15] Step 1 is catalyzed by copper salts, as were similar reactions that were described in the past.[1,2,3] In the absence of a copper salt, the reaction proceeds at a much slower rate. The pyrolysis in step 2 occurs at 80–100°, whereas temperatures of 250–400° were employed

[12] M. S. Kharasch and G. Sosnovsky, Abstracts of Papers presented at the 134th Meeting of the American Chemical Society, Chicago, Sept. 1958, p. 7P.
[13] D. D. Denney, D. Z. Denney and G. Feig, *Tetrahedron Letters* No. 15, 19 (1959).
[14] J. Hine, *Physical Organic Chemistry* p. 462. McGraw-Hill, New York (1956).
[15] D. J. Gramm and M. S. Newman, *Steric Effects in Organic Chemistry* p. 305. John Wiley, New York (1956).

in previous studies of related substances.[10] The addition of alcohols to olefins is usually accomplished in the presence of mineral acids.[9,10] Therefore, it was surprising to find that even acetic and benzoic acids acted as catalysts in the addition reaction in step 3.

EXPERIMENTAL

t-Butyl perbenzoate and t-butyl peracetate were obtained from Lucidol Division, Wallace and Tiernan, Inc. They were used without further purification. The cuprous bromide was an anhydrous, reagent-grade product. All the experiments were carried out in a nitrogen atmosphere. The reaction vessel was equipped with a stirrer and a reflux condenser and was connected to a gas meter. No gases were observed in the work reported. The boiling points are uncorrected. The molecular weights were determined cryoscopically in benzene. For further details see preceding publications.

Reactions of peresters with monofunctional ethers

t-Butyl perbenzoate with n-propyl ether. t-Butyl perbenzoate (39 g, 0·2 mole) was added over a period of 2 hr to a mixture of n-propyl ether (37 g, 0·37 mole) and cuprous bromide (0·1 g, 0·35 mmole) maintained at 85–95°. After 4 hr of heating, the cooled reaction mixture was diluted with ether (50 ml) and extracted with 2 N sodium carbonate to remove benzoic acid (10·2 g). The ethereal solution was washed with water, dried with anhydrous sodium sulfate, and concentrated at 10 mm. The remaining oil was distilled at reduced pressure. There were obtained: 1-*t-butoxy*-1-*n-propoxypropane* (IVa, 6 g, 17%), b.p. 60–62°/20 mm, n_D^{25} 1·4032, and 1-*benzoyloxy*-1-*n-propoxypropane* (I, 17 g, 38·5%), b.p. 88–92°/0·04 mm (decomp.), ν_{max} 1730 cm^{-1} (C=O).

t-Butyl alcohol (50 ml) was added to the benzoyloxy derivative, and the reaction mixture was heated at reflux for 17 hr. Following the usual workup, there were obtained: benzoic acid (6 g, 65%), unreacted benzoyloxy compound (2·6 g), and 1-t-butoxy-1-n-propoxypropane (IVa, 4·5 g, 35%), b.p. 52°/10 mm, n_D^{25} 1·4030. (Found: C, 68·44; H, 12·77; mol. wt., 166. $C_{10}H_{22}O_2$ requires: C, 68·91; H, 12·72%; mol wt., 174).

t-Butyl perbenzoate with n-butyl ether. t-Butyl perbenzoate (19·4 g, 0·1 mole) was added over a period of 4 hr to a mixture of n-butyl ether (52 g, 0·4 mole) and cuprous bromide (0·1 g, 0·35 mmole) maintained at 95°. Heating was continued for 10 hr after the addition of t-butyl perbenzoate. The cooled reaction mixture was diluted with ethyl ether (50 ml) and extracted with 2 N sodium carbonate to remove benzoic acid (6·8 g, 56·5%). The ethereal solution was washed with water, dried with anhydrous sodium sulfate, and concentrated at 10 mm. The remaining oil was distilled at reduced pressure. There were obtained: n-butyl ether (28 g), b.p. 42°/17 mm, 1-*n-butoxy*-1-*t-butoxybutane* (IVb, 5·5 g, 27·5%), b.p. 88–90°/10 mm, n_D^{25} 1·4148, and 1-*benzoyloxy*-1-*n-butoxybutane* (I, 10 g, 40%), b.p. 80°/0·04 mm (some decomp.).

To the benzoyloxy derivative was added t-butyl alcohol (40 ml), and the reaction mixture was heated at reflux for 48 hr. Following the usual workup, there were obtained: benzoic acid (4·8 g) and 1-n-butoxy-1-t-butoxybutane (IVb, 3·1 g, 39%), b.p. 88–90°/10 mm, n_D^{25} 1·4148. (Found: C, 71·15; H 12·65; mol. wt., 194. $C_{12}H_{26}O_2$ requires: C, 71·2; H, 12·96%; mol. wt., 202).

t-Butyl peracetate with tetrahydrofuran. A mixture of tetrahydrofuran (133 g, 1·85 moles), t-butyl peracetate (150 ml of a 75% benzene solution, 0·8 mole), and cuprous bromide (0·1 g, 0·35 mmole) was heated under reflux for 14 hr. The cooled reaction mixture was diluted with ether (50 ml) and extracted with dilute sodium carbonate to remove acetic acid. The ethereal solution was washed with water, dried with sodium sulfate, and concentrated on a steam bath. The remaining liquid was distilled at reduced pressure. There were obtained: a small amount of 2-acetoxytetrahydrofuran II, 4 g), b.p. 34–40°/0·1 mm, and 2-*t-butoxytetrahydrofuran* (V, 46 g, 45%), b.p. 40–42°/13 mm, n_D^{25} 1·4186, ν_{max} 1390, 1365 cm^{-1} (—C(CH$_3$)$_3$), no bands at 755, 695 cm^{-1} (C_6H_5—), 1740, 1730 cm^{-1} (C=O), and in the hydroxy region. (Found: C, 66·78; H, 11·23; mol. wt., 146. $C_8H_{14}O_2$ requires: C, 66·80; H, 11·02%; mol. wt., 144).

t-Butyl perbenzoate with tetrahydrofuran. A mixture of t-butyl perbenzoate (0·5 mole), tetrahydrofuran (265 g, 3·7 moles), and cuprous bromide (0·1 g, 0·35 mmole) was heated under reflux for 14 hr. The cooled reaction mixture was diluted with ether (50 ml) and extracted with 2 N sodium carbonate solution to remove benzoic acid (41 g). The ethereal solution was washed with water, dried

[10] C. H. DePuy, R. W. King and D. H. Froemsdorf, *Tetrahedron* **7**, 123 (1959).

with sodium sulfate, and concentrated on a steam bath. The remaining oil was distilled at reduced pressure. There was obtained: 2-t-butoxytetrahydrofuran (V, 32 g, 45%), b.p. 40°/11mm, n_D^{25} 1·4186, ν_{max} at 1390, 1365 cm^{-1} (—$C(CH_3)_3$), no bands at 755, 695 cm^{-1} (C_6H_5—), 1740, 1730 cm^{-1} (C=O), and in the hydroxy region. (Found: C, 66·78; H, 11·23; mol. wt., 146. $C_8H_{16}O_2$ requires: C, 66·80; H, 11·02%; mol. wt., 144). In addition, there was obtained: *2-benzoyloxytetrahydrofuran* (II, 25 g, 26%), b.p. 96–100°/0·2 mm (some decomp.), ν_{max} at 1740 cm^{-1} (C=O). Due to its instability, this compound could not be purified sufficiently for elemental analysis, but its infrared analysis and the fact that its thermal decomposition led to the formation of the corresponding acid removed all doubt that this compound is the acyloxy derivative.

t-Butyl perbenzoate with tetrahydrofuran in the absence of cuprous bromide. A mixture of tetrahydrofuran (89 g, 1·22 moles) and t-butyl perbenzoate (39 g, 0·2 mole) was heated under reflux for 48 hr. The cooled reaction mixture was diluted with ether (50 ml) and extracted with dilute sodium carbonate to remove benzoic acid (16 g, 67%). The ethereal solution was washed with water, dried with sodium sulfate, and concentrated on a steam bath. The remaining oil was distilled at reduced pressure. There were obtained: 2-t-butoxytetrahydrofuran (V, 6 g, 21%), b.p. 36–40°/10 mm, n_D^{25} 1·4190, ν_{max} identical with those of an authentic sample, unreacted t-butyl perbenzoate (6·5 g, 6·8%), b.p. 68–70°/0·05–0·1 mm, n_D^{25} 1·5008, and a residue (4 g) consisting of a mixture of t-butyl perbenzoate and 2-benzoyloxytetrahydrofuran (II).

t-Butyl perbenzoate with tetrahydropyran. t-Butyl perbenzoate (78 g, 0·4 mole) was added over a period of 2 hr to a mixture of tetrahydropyran (86 g, 1 mole) and cuprous bromide (0·1 g, 0·35 mmole) maintained at 84°. After the addition of t-butyl perbenzoate, the reaction mixture was heated under reflux for 10 hr. The cooled reaction mixture was diluted with ether (50 ml) and extracted with 2 N sodium carbonate to remove benzoic acid (34·5 g, 72%). The ethereal solution was washed with water, dried with sodium sulfate, and concentrated at 10 mm. The remaining oil was distilled at reduced pressure. There were obtained: *2-benzoyloxytetrahydropyran* (III, 20 g, 24%), which pyrolyzed on attempted distillation at 0·1 mm, and *2-t-butoxytetrahydropyran* (VI, 21 g, 33%), b.p. 56°/13 mm, n_D^{25} 1·4268, ν_{max} 1390, 1365 cm^{-1} (—$C(CH_3)_3$), no bands at 755, 695 cm^{-1} (C_6H_5—), 1720 cm^{-1} (C=O), and in the hydroxy region. (Found: C, 68·65; H, 11·61; mol. wt., 165. $C_9H_{16}O_2$ requires: C, 68·30 H, 11·48%; mol. wt., 158).

Decomposition of 2-benzoyloxytetrahydrofuran

2,3-Dihydrofuran. 2-Benzoyloxytetrahydrofuran (11·6 g, 0·06 mole) was heated over a period of 1 hr to 130–135°. The product was collected in a trap, which was cooled by a Dry Ice–isopropanol mixture. After repeated distillation, 2,3-dihydrofuran (2·8 g, 66%) was obtained, b.p. 52–54°/747 mm, n_D^{25} 1·4200 (lit. values:[7] b.p. 54°/764 mm, n_D^{20} 1·4230). The pot residue was identified as benzoic acid (5·3 g, 69%).

Reaction of dihydropyran with t-butyl alcohol

In the presence of benzoic acid. A mixture of dihydropyran (24 g, 0·285 mole), benzoic acid (20 g, 0·165 mole), and t-butyl alcohol (27 g, 0·365 mole) was heated under reflux for 24 hr. The cooled reaction mixture was diluted with ether and extracted with 2 N sodium carbonate solution to remove benzoic acid (14 g). The ethereal solution was washed with water, dried with sodium sulfate, and concentrated on a steam bath. The remaining oil was distilled at reduced pressure. There were obtained: 2-t-butoxytetrahydropyran (VI, 17·5 g, 39%), b.p. 52°/11 mm, n_D^{25} 1·4268, and a residue (7·0 g).

The residue was diluted with a fourfold amount of t-butyl alcohol, and the mixture was heated under reflux for 48 hr. After the usual workup, there were obtained: benzoic acid (2·8 g, 66%) and 2-t-butoxytetrahydropyran (VI, 3 g, 53%) b.p. 52–54°/12 mm, n_D^{25} 1·4268, ν_{max} identical with those of an authentic sample prepared by the method of Paul[9,10] from molar quantities of t-butyl alcohol, dihydropyran, and catalytic amounts of concentrated hydrochloric acid.

In the presence of acetic acid. A mixture of dihydropyran (24 g, 0·285 mole), glacial acetic acid (20 g, 0·3 mole), and t-butyl alcohol (27 g, 0·365 mole) was heated at reflux for 12 hr. After the usual workup, there was obtained: 2-t-butoxytetrahydropyran (VI, 20 g, 44·5%), b.p. 56°/13 mm, n_D^{25} 1·4270, ν_{max} identical with those of an authentic sample.

There was no reaction between dihydropyran and t-butyl alcohol when acid was excluded from the system.

In the presence of cuprous bromide. A mixture of dihydropyran (24 g, 0·285 mole), t-butyl alcohol (27 g, 0·365 mole), and cuprous bromide (0·1 g, 0·35 mmole) was heated under reflux for 12 hr. After the usual workup, there was obtained: 2-t-butoxytetrahydropyran (VI, 3·5 g, 7·8%), b.p. 53°/12 mm, n_D^{25} 1·4272, ν_{max} identical with those of an authentic sample.

Reactions of t-butyl perbenzoate with difunctional ethers

With dioxane. Dioxanyl benzoate was prepared from a mixture of dioxane (0·5 mole), t-butyl perbenzoate (0·2 mole), and cuprous bromide (0·35 mmole).[3] Without isolating the benzoate, the reaction mixture was heated for 114 hr at 105–110°. The reaction mixture was then distilled at atmospheric pressure with a short, tungsten, spiral column (10 plates). There were obtained: t-butyl alcohol (6 g, 40%) and dioxane (24 g, 0·23 mole).

The residue was diluted with ether (50 ml) and extracted with 2 N sodium carbonate to remove benzoic acid (15 g, 62%). The ethereal solution was concentrated on a steam bath, and the remaining oil was distilled under reduced pressure. There was obtained: 2-*t-butoxy*-1,4-*dioxane* (IX, 6 g, 19%), which was distilled repeatedly to a constant refractive index, b.p. 51–52°/3 mm, n_D^{25} 1·4288. (Found: C, 59·23; H, 9·79; mol. wt., 156. $C_8H_{16}O_2$ requires: C, 59·98; H, 10·07%; mol. wt., 160).

Distillation of the residue gave unreacted dioxanyl benzoate (12·5 g, 30%), b.p. 106°/0·2 mm,[3] and a higher boiling material (5 g).

With 1,2-dimethoxyethane. t-Butyl perbenzoate (20 g, 0·1 mole) was added over a period of 4 hr to a mixture of 1,2-dimethoxyethane (0·6 mole) and cuprous bromide (0·35 mmole) at 90°. An exothermic reaction resulted from each addition of perbenzoate. The usual workup gave benzoic acid (1·3 g, 10%). The remaining oil was distilled at reduced pressure. There was obtained: 1-*benzoyloxy*-1,2-*dimethoxyethane* (VIII, 14 g, 67%), b.p. 94–98°/0·1 mm, n_D^{25} 1·4859, ν_{max} 1730 cm^{-1} (C=O). Due to some decomposition of the product during distillation, it could not be purified sufficiently for elemental analysis.

t-Butyl perbenzoate (50 g, 0·25 mole) was added to a mixture of 1,2-dimethoxyethane (1·0 mole) and cuprous bromide (0·35 mmole) at 90° over a period of 2 hr. After the addition of the perbenzoate, t-butyl alcohol (25 ml) was poured into the reaction mixture and the heating was continued at 100–105° for 115 hr. The cooled reaction mixture was washed with 2 N sodium carbonate to remove benzoic acid (16 g, 52%). After distillation of the remaining oil under reduced pressure, there was obtained: 1-*t-butoxy*-1,2-*dimethoxyethane* (X, 3 g, 7·5%), b.p. 38–42°/1·2 mm, n_D^{25} 1·4092. (Found: C, 59·43; H, 11·25; mol. wt., 166. $C_8H_{18}O_3$ requires: C, 59·23; H, 11·18%; mol. wt., 169).

After further distillation of the residue, there was obtained: 1-t-benzoyloxy-1,2-dimethoxyethane (VIII, 13 g, 25%), b.p. 100°/0·4 mm, n_D^{25} 1·4848, ν_{max} identical with that of the sample prepared in the preceding experiment.

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DIE UMSETZUNG VON KETOHEXOSEN MIT SEKUNDÄREN AMINOSÄUREN UND SEKUNDÄREN AMINEN[1]

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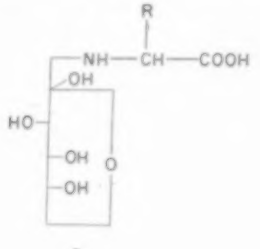
Zusammenfassung—Die Umsetzung von D-Fructose mit L-Prolin liefert 2-Desoxy-2-N-L-prolino-D-glucose neben 2-Desoxy-2-N-L-prolino-D-mannose und 1-Desoxy-1-N-L-prolino-D-fructose, die auch durch direkte Reaktion von D-Glucose mit L-Prolin erhältlich ist. Die Umsetzung von L-Sorbose mit L-Prolin liefert 1-Amino-1-N-L-prolino-L-sorbose und nur sehr wenig Reaktionsprodukte der Ketosylamin-Umlagerung.

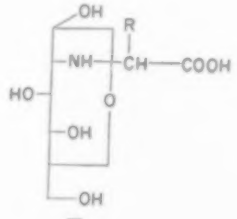
Durch Einwirkung von Piperidin wird D-Fructose in hohem Masse zu D-Glucose, durch Morpholin, Dicyclohexylamin und andere Amine zu D-Psicose isomerisiert. Diese Reaktion ist zur präparativen Darstellung von D-Psicose geeignet.

BEI der Umsetzung von Hexosen mit Aminosäuren lassen sich zwei unterschiedliche Reaktionstypen unterscheiden. Aldosen reagieren mit Aminosäuren primär zu N-Glycosiden, den äusserst labilen Aldosylaminosäuren, die sehr leicht unter dem katalytischen Einfluss der in der Aminosäure vorhandenen Carboxylgruppe einer Amadori-Umlagerung (Aldosylamin-Umlagerung) unterliegen, wobei Verbindungen vom Typ I gebildet werden.[2-4] Aus D-Glucose und L-Alanin entsteht auf diesem Wege 1-Desoxy-1-N-L-alanino-D-fructose (I, R = CH_3), welche abgekürzt als Fructose-L-alanin bezeichnet wird. Eine Reihe derartiger "Fructose-Aminosäuren" einer grossen Zahl von Aminosäuren ist dargestellt worden.[2,3]



I

Fructose – Aminosäure
aus Glucose + Aminosäure



II

Glucose – Aminosäure
aus Fructose + Aminosäure

Ketosen reagieren gleichfalls mit Aminosäuren unter intermediärer Bildung von N-Glycosiden, die mittels Säurekatalyse auf dem Wege einer analogen Ketosylamin-Umlagerung zu Verbindungen vom Typ II umlagern. So entsteht aus D-Fructose und

[1] Vorhergehende Mitt. K. Heyns und M. Rolle, Chem. Ber. **92**, 2439, 2451 (1959).
[2] A. Abrams, P. H. Lowy und H. Borsook, *J. Amer. Chem. Soc.* **77**, 4794 (1955); P. H. Lowy und H. Borsook, *Ibid.* **78**, 3175 (1956); A. Klemer und F. Micheel, *Chem. Ber.* **89**, 1242 (1956); F. Micheel und A. Frowein, *Ibid.* **92**, 304 (1959).
[3] K. Heyns und H. Paulsen, *Liebigs Ann.* **622**, 160 (1959).
[4] E. F. L. J. Anet, *Austr. J. Chem.* **10**, 193 (1957); E. F. L. J. Anet und T. M. Reynolds, *Ibid.* **10**, 182 (1957).

L-Alanin 2-Desoxy-2-N-L-alanino-D-glucose II ($R = CH_3$), abgekürzt "Glucose-L-alanin". Auch hier sind mehrere Vertreter dieser "Glucose-Aminosäuren" dargestellt worden.[1,5] Die Ketosylamin-Umlagerung, die von Heyns und Koch[6] an der Umsetzung von D-Fructose mit Ammoniak unter Bildung von D-Glucosamin im Jahre 1952 aufgefunden wurde, ist im Gegensatz zur Aldosylamin-Umlagerung mit der Ausbildung eines neuen Asymmetriezentrums verbunden, das am C-Atom 2 gebildet werden kann. Aus diesem Grunde werden neben den Glucose-Aminosäuren je nach den Reaktionsbedingungen in wechselnden Mengen Mannose-Aminosäuren erhalten. Beide Reaktionstypen haben sich bisher mit einer grossen Anzahl verschiedenster Aminosäuren, die eine primäre Aminogruppe aufweisen, an einer Reihe von Aldosen und Ketosen durchführen lassen. Diese Umsetzungen stellen den wichtigen Primärschritt für die als Maillard-Reaktion bezeichneten nichtenzymatischen Bräunungsreaktionen dar.[7] Bezüglich Entstehung bzw. Vorhandenseins von Hexose-Aminosäuren in Leberextrakten sei auf unsere letzte Mitteilung hierüber verwiesen.[3]

Umsetzung von D-*Fructose-mit* L-*Prolin*

In der vorliegenden Untersuchung werden die Reaktionen von Fructose mit sekundären Aminen und sekundären Aminosäuren behandelt. Glucose lässt sich mit sekundären Aminen umsetzen, wie bereits Hodge und Rist[8] gezeigt haben. Sie erhielten mit Piperidin das entsprechende N-Glucosid, welches unter Amadori-Umlagerung in 1-Desoxy-1-piperidino-D-fructose überführbar war. Diese Versuche zeigen, dass als Ausgangsprodukte der Amadori-Umlagerung stets die N-Glycoside angesehen werden müssen und kaüm offene Formen nach Art der Schiff' schen Basen. Auch mit aromatischen sekundären Aminen und 4,6-Benzalglucose ist nach Weygand *et al.*[9] eine Umlagerung zu 1-Desoxy-1-Amino-D-fructose-Verbindungen möglich.

N-Glucoside von sekundären Aminen sind allgemein leichter spaltbar als entsprechende Verbindungen von primären Aminen und lassen sich schwieriger umlagern. Dies zeigte sich erwartungsgemäss bei der Umsetzung von L-Prolin mit Fructose, deren N-Fructoside wiederum leichter als N-Glucoside spaltbar sind. L-Prolin setzt sich wesentlich schwieriger um als alle vorher untersuchten Aminosäuren mit primärer Aminogruppe.[1,5] Unter extrem wasserfreien Bedingungen wurde in Methanol ein Gemisch von 3 Hexoseaminosäuren in einer Gesamtausbeute von nur etwa 10% erhalten. Die Bildung des primär unter Wasserabspaltung entstehenden leicht spaltbaren N-Fructosyl-L-prolin (IV) führt offenbar zu einem Gleichgewicht, welches sehr zu Ungunsten des N-Fructosids liegt. Eine Ketosylamin-Umlagerung findet, wie bei den anderen Aminosäuren, ohne Säurezusatz statt, da bereits die Carboxylgruppe des Prolins die Umlagerung katalysiert.

Als störende Nebenreaktion tritt eine vielfältige Aufspaltung des L-Prolins auf. L-Prolin, welches unter vergleichbaren Bedingungen äusserst stabil ist, erleidet bei Gegenwart von Fructose sowohl in Methanol, Dimethylsulfoxyd und Wasser eine Reihe noch nicht aufgeklärter Umwandulungs- und Zersetzungsreaktionen. So treten

[5] K. Heyns, H. Breuer und H. Paulsen, *Chem. Ber.* **90**, 1374 (1957); K. Heyns und H. Breuer, *Ibid.* **91**, 2750 (1958).
[6] K. Heyns und W. Koch, *Z. Naturf.* **7b**, 486 (1952).
[7] K. Heyns und H. Paulsen, *Wissenschaftl. Veröffentlichungen der Deutschen Gesellschaft für Ernährung* Bd. 5, 15. Steinkopff-Verlag, Darmstadt (1960).
[8] J. E. Hodge und C. E. Rist, *J. Amer. Chem. Soc.* **75**, 316 (1953).
[9] F. Weygand, H. Simon und R. v. Ardenne, *Chem. Ber.* **92**, 3117 (1959).

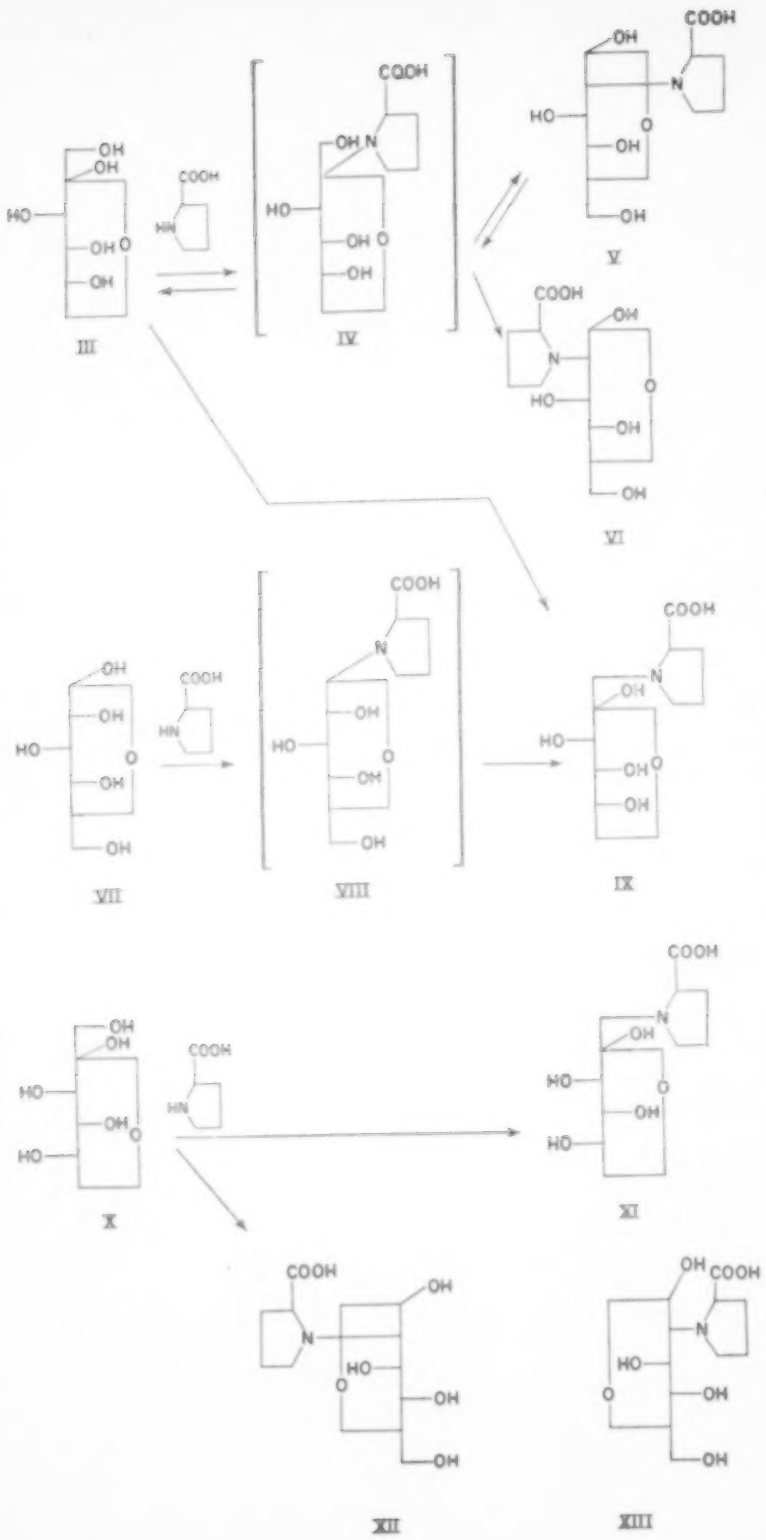
auch unter mildesten Bedingungen (37°) bei der Einwirkung von Fructose auf L-Prolin in kurzer Zeit vier neue Ninhydrin-positive (drei blau anfärbbar, eine gelb anfärbbar) Substanzen auf, deren Anzahl bei längerer Einwirkungszeit sich noch vermehrt. Diese Zersetzungsreaktion tritt ausschliesslich nur mit Fructose auf. Gegenüber anderen Zuckern, wie D-Glucose und L-Sorbose, is L-Prolin stabil.

Die durch Ketosylamin-Umlagerung entstandenen Hexose-Aminosäuren wurden mittels saurem Austauscher abgetrennt und an Austauschersäulen mehrfach fraktioniert. Es wurden zwei einheitliche kristallisierte Verbindungen erhalten, die sich als 2-Desoxy-2-N-prolino-D-glucose (D-Glucose-L-prolin, V) und 1-Desoxy-1-N-L-prolino-D-fructose (D-Fructose-L-prolin, IX) erwiesen. Die zu erwartende dritte, nur in geringer Menge gebildete Substanz, das D-Mannose-L-prolin (VI), liess sich nicht völlig rein erhalten. Das ungefähre Mengenverhältnis der drei entstandenen Hexose-Aminosäuren beträgt: D-Glucose-L-prolin 30–40%, D-Mannose-L-prolin 5–10%, D-Fructose-L-prolin 40–60%. Alle drei Substanzen sind papierchromatographisch trennbar. Die Glucose- und Mannose-Verbindungen geben mit Silbernitrat einen hellbraunen, die Fructose-Verbindungen den für Amadori-Verbindungen typischen schwarzbraunen Fleck. D-Glucose-L-Prolin wird entgegen den bisherigen Erfahrungen bei anderen Glucose-Aminosäuren nicht als erste, sondern als letzte Substanz bei der Säulenchromatographie an saurem Austauscher eluiert.[5]

D-Glucose-L-prolin (V) liess sich auf Grund der optischen Drehung in seiner Konfiguration zuordnen. Die Substanz besitzt eine optische Drehung von $[\alpha]_D^{20} + 2°$, welche erheblich niedriger als die der anderen Glucose-Aminosäuren liegt, die einen Drehwert zwischen $[\alpha]_D^{20} +48°$ bis $+81°$ aufweisen. Die niedrige optische Drehung ist offenbar durch den hohen negativen Drehwertanteil des L-Prolins von $[\alpha]_D^{20} -85°$ bedingt, welcher additiv den positiven Anteil der Kohlenhydratgruppe überlagert. Durch Anwendung der Regel von Bose und Chatterjee[10] lässt sich folgern, dass Mannose-Aminosäuren gegenüber Glucose-Aminosäuren stets eine negativere optische Drehung haben müssen. Dies ist bei den bisher dargestellten Mannose-Aminosäuren, welche einen Drehwert zwischen $[\alpha]_D^{20} -7°$ bis $-16°$ besitzen, stets bestätigt gefunden. Die weitgehend angereicherten Fraktionen des sirupös erhaltenen vermeintlichen Mannose-L-prolins, welche möglichst frei von stark negativ drehenden D-Fructose-L-prolin (IX) waren, zeigten gleichfalls eine negative optische Drehung von $[\alpha]_D^{20} -25°$. Damit ist erwiesen, dass die kristallisierte Substanz eine D-Gluco-, die als Sirup erhaltene Substanz eine D-Manno-Konfiguration besitzt. D-Glucose-L-prolin zeigt beim Auflösen in H_2O eine dextrorotäre Mutarotation. Die Verbindung liegt in kristallisierter Form demnach in der β-Konfiguration vor. Alle anderen Glucose-Aminosäuren kristallisierten in der α-Form.

Überraschend ist der mengenmässig hohe Anteil des aus Fructose und L-Prolin gebildeten D-Fructose-L-prolins (IX), welches bei der Umsetzung von Fructose mit Prolin über die Ketosylamin-Umlagerung nicht zu erwarten ist. Wir haben die gleiche Substanz auf anderem Wege durch direkte Umsetzung von D-Glucose mit L-Prolin, also über die Aldosylamin-Umlagerung, erhalten. Glucose (VII) setzte sich in Methanol bei 60° sehr schnell und in hoher Ausbeute mit L-Prolin um, wobei unter Amadori-Umlagerung des intermediär gebildeten Glucosyl-L-prolins (VIII) D-Fructose-L-prolin (IX) entsteht, welches leicht in kristalliner Form isoliert werden konnte. Die Verbindung zeigt die charakteristischen Reduktionsreaktionen für Amadori-Verbindungen

[10] A. K. Bose und B. G. Chatterjee, *J. Org. Chem.* **23**, 1425 (1958).


III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII

und ist in allen Eigenschaften identisch mit der auch aus Fructose und L-Prolin erhaltenen zweiten kristallinen Hexose-Aminosäure.

D-Glucose-L-prolin (V) ist in stark saurer Lösung stabil. In schwach saurer Lösung erfolgt verhaltnismässig leicht eine Rückumlagerung[6] zum N-Fructosid (IV), welches sofort in die Ausgangskomponenten gespalten wird. So wird durch Erhitzen von D-Glucose-L-prolin (V) in verdünnter Essigsäure Prolin, Fructose und Hydroxymethylfurfurol erhalten. Diese Rückspaltung erfolgt bei p_H 4,2 bereits bei Zimmertemperatur in einigen Tagen. Sie steht in gewisser Analogie zu der von Anet[11] aufgefundenen Rückspaltung von Di-D-fructose-glycin, einer Verbindung, die gleichfalls ein tertiäres Stickstoffatom am Kohlenhydratrest besitzt. Bei p_H 5,5 in wässriger Lösung bei Zimmertemperatur erleidet sie eine Rückspaltung in D-Fructose-glycin und 3-Desoxy-D-erythrohexoson, einer Verbindung, die sich leicht in saurer Lösung in Hydroxymethylfurfurol umwandeln kann. Glucose tritt als Spaltprodukt dieser Reaktion, wie bei jeder Rückspaltung von Fructose-Aminosäuren,[7] nicht auf. Auch die Rückspaltung von D-Fructose-L-prolin in verdünnter Essigsäure liefert nur L-Prolin und Hydroxymethylfurfurol.

Als weitere Ketose haben wir L-Sorbose (X) mit L-Prolin umgesetzt. Die Reaktion gelingt nur in extrem wasserfreiem Dimethylsulfoxyd und führt zu zwei Hexose-Aminosäuren. Die Säulenauftrennung lieferte in weitaus grösster Menge als Hauptprodukt nicht die durch Ketosylamin-Umlagerung entstehenden Verbindungen, sondern kristallisiertes L-Sorbose-L-prolin (XI), welches normalerweise durch Amadori-Umlagerung aus L-Gulose oder L-Idose mit L-Prolin entstehen sollte. Die Verbindung reagiert als Amadori-Verbindung positiv mit alkalischer $K_3[Fe(CN)_6]$-Lösung, ihre Spaltung mit verdünnter Essigsäure liefert nur L-Prolin und Hydroxymethylfurfurol und keine L-Sorbose, die aus einer Aldose-Aminosäure zu erwarten ist. Aus den Mutterlaugen wurde eine amorphe Substanz erhalten, die ein Gemisch aus L-Sorbose-L-prolin und einer erwartungsgemäss durch Ketosylamin-Umlagerung entstandene Aldose-Aminosäure darstellt. Die Rückspaltung dieser Substanz mit verdünnter Essigsäure liefert neben L-Prolin und Hydroxymethylfurfurol auch L-Sorbose, was einwandfrei für das Vorliegen einer Aldose-Aminosäure spricht. Es handelt sich vermutlich um L-Gulose-L-prolin (XII) und nicht L-Idose-L-prolin (XIII), da nach unseren Erfahrungen bei der Umsetzung von L-Sorbose mit Glycin bevorzugt L-Gulose-glycin gebildet wird.

L-Hydroxyprolin liess sich weder mit Fructose noch mit Sorbose zu den entsprechenden Hexose-Aminosäuren umsetzen. Dies Ergebnis stimmt mit den Beobachtungen bei aliphatischen Aminosäuren überein. Auch dort reagieren Oxyaminosäuren z.B. L-Serin, schwieriger als L-Alanin.[5]

In den IR-Spektren von D-Glucose-L-prolin, D-Fructose-L-prolin und L-Sorbose-L-prolin lassen sich keine Carbonylbanden nachweisen. Es liegen demnach auch die Ketose-L-prolin-Verbindungen in cyclischer Halbacetalform vor. Sie stimmen in ihrer Struktur durchaus mit anderen Ketose-Aminosäuren und mit 1-Desoxy-piperidino-D-fructose überein. Offenkettige Amadori-Verbindungen mit sekundären Aminen liegen vor in den 1-Desoxy-(1-methylarylamino)D-fructosen.[9] Ebenfalls offenkettig sind nach Heyns und Schulz[12] die Fructuron-Aminosäuren.

Die Bildung von D-Glucose-L-prolin (V) und D-Mannose-L-prolin (VI) aus Fructose

[11] E. F. L. J. Anet, *Austr. J. Chem.* **12**, 280 (1959); *J. Amer. Chem. Soc.* **82**, 1502 (1960).
[12] K. Heyns und W. Schulz, *Chem. Ber.* **93**, 128 (1960).

(III) und L-Prolin verläuft über ein intermediär gebildetes N-Fructosid (IV), welches eine normale Ketosylamin-Umlagerung eingeht. Das Mengenverhältnis von V und VI wird offenbar durch sterische Effekte bestimmt, da es durch Variation des Lösungsmittels nicht wesentlich zu beeinflussen ist. Das Kalotten-Modell lässt erkennen, dass axial-ständiges Prolin im D-Mannose-L-prolin (VI) stärker behindert ist, als äquatorial-ständiges Prolin im D-Glucose-L-prolin (V), so dass das Mengenverhältnis der Umlagerungsprodukte zu Gunsten der letzteren Verbindung verschoben sein dürfte, wie es auch gefunden wurde.

Schwieriger ist die Bildung von D-Fructose-L-prolin (IX) aus Fructose und L-Prolin zu erklären, da diese Verbindung normalerweise nur aus Glucose (VII) und L-Prolin über eine Amadori-Umlagerung des entsprechenden N-Glucosids (VIII) entstehen kann. In früheren Modellversuchen an entständigen Ketolen mit der Gruppierung R—CO—CH_2OH konnten wir zeigen,[13] dass diese Verbindungen mit Aminen in der Regel aus der Form ihrer isomeren Hydroxyaldehyde unter anschliessender Umlagerung zu ω-Aminoketonen und nicht zu α-Aminoaldehyden reagieren. Es setzen sich hierbei die im Gleichgewicht vorhandenen geringen Hydroxyaldehydanteile infolge ihrer höheren Reaktivität in Richtung zum ω-Aminoketon um. Da bekanntlich ein sehr geringer Anteil der offenen Form der Fructose stets im Gleichgewicht mit der Halbacetalform vorliegt, wäre eine Konkurrenzreaktion der offenen Fructose im Sinne der obigen Modellversuche zu diskutieren, die dann zu Fructose-Aminosäuren führen müsste. Glucose liess sich allerdings in derartigen Reaktionslösungen im Sinne eines vorgelagerten Gleichgewichts- oder Umlagerungsproduktes niemals nachweisen. Als zweiter Mechanismus der Fructose-L-prolin-Bildung wäre eine Sekundärreaktion von Glucose-L-Prolin mit überschüssigem L-Prolin in Betracht zu ziehen. Glucose-L-prolin kann mit L-Prolin, wie wir fanden, am C-Atom 1 zu einem N-Glycosid reagieren, welches einer Amadori-Umlagerung zu einem Umlagerungsprodukt unterliegt, dessen Spaltung dann Fructose-L-prolin liefert. So lässt sich beim Erhitzen von Glucose-L-prolin in Methanol mit L-Prolin die Bildung von Fructose-L-prolin, beim Erhitzen mit L-Valin gewissermassen im Kreuzeversuch die Bildung von Fructose-L-Valin nachweisen. Ein derartiger Reaktionsverlauf lässt sich auch bei anderen Glucose-Aminosäuren zeigen, z.B. gibt Glucose-L-Valin durch Umsetzung mit L-Valin Fructose-L-Valin. Über das Ausmass beider nebeneinander verlaufenden Reaktionswege können nur quantitative Untersuchungen Aufschluss geben.

Das Verhältnis der entstehenden Glucose- und Fructose-Aminosäuren ist durch die Bildungsgeschwindigkeit der Glucose-Aminosäuren bestimmt. Werden diese sehr rasch gebildet, so wird die angebotene Aminosäure schnell zur Glucose-Aminosäure umgesetzt und Nebenreaktionen treten kaum ein Dies entspricht in der Tat den Beobachtungen. Mit ω-ständigen Aminosäuren, wie z.B. β-Alanin, γ-Aminobuttersäure und ε-Aminocapronsäure, lässt sich die Ketosylamin-Umlagerung sehr leicht bereits bei Zimmertemperatur durchführen. Die Konkurrenzreaktion kommt hier kaum zum Tragen und es werden nur untergeordnete Mengen von Fructose-Aminosäure neben Aldose-Aminosäuren gefunden. Je grösser der Rest der Seitenkette bei einer α-Aminosäure ist, desto mehr wird die Ketosylamin-Umlagerung erschwert. Dies ist bei L-Valin, insbesondere aber bei L-Phenylalanin, L-Asparaginsäure und L-Glutaminsäure der Fall. Diese Aminosäuren liefern daher bei der Reaktion mit Fructose neben Aldose-Aminosäuren einen betrachtlichen Anteil an Fructose-Aminosäuren. Bei der

[13] K. Heyns und W. Stumme, *Chem. Ber.* **89**, 2833, 2844 (1956).

Umsetzung mit Prolin ist durch die sekundäre Aminogruppe die Ketosylamin-Umlagerung weiterhin erschwert, wodurch sich die durch Nebenreaktion gebildete Menge an Fructose-Aminosäure auf über die Hälfte der gesamten Reaktionsprodukte erhöht.

Mit Sorbose lässt sich, wie wir von den Umsetzungen mit Ammoniak wissen,[14] die Ketosylamin-Umlagerung erheblich schwerer durchführen als mit Fructose. Bei der Reaktion von Sorbose mit L-Prolin wird demnach die Ketosylamin-Umlagerung zusätzlich von der Kohlenhydratkomponente her erschwert. Dies hat zur Folge, dass die Nebenreaktion zur Ketose-Aminosäure zur Hauptreaktion wird und in überwiegender Menge L-Sorbose-L-prolin neben nur wenig Aldose-L-prolin erhalten wird.

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Reaktion von Fructose mit sekundären Aminen

Nach Heyns u. Mitarbeitern und Carson[15,16] reagieren primäre Amine bei 0° glatt mit Fructose, wobei unter Ketosylamin-Umlagerung der primär gebildeten N-Fructoside N-substituierte Derivate des Glucosamins entstehen. Mit sekundären Aminen konnten wir unter vergleichsweisen Bedingungen mit Fructose keine Reaktion feststellen.

Bei erhöhten Temperaturen (40–60°) liess sich durch Umsetzung von Fructose mit Piperidin ein sirupöses, durch Säure leicht spaltbares N-Glycosid gewinnen. Dieses konnte mit Essigsäure als Katalysator in guter Ausbeute zu einem säurestabilen Amino-zuckergemisch umgelagert werden, das im wesentlichen aus zwei Substanzen bestand, von denen die eine Komponente in weitaus grösserer Menge gebildet worden war. Die Reaktionslösung enthielt neben Fructose in grossen Mengen durch Lobry de Bruyn-van Ekenstein-Umlagerung unter dem Einfluss des stark basischen Piperidins entstandene Glucose.

Das mittels Austauschersäulenfraktionierung abgetrennte Hauptprodukt erwies sich als identisch mit der von Hodge und Rist[8] beschriebenen 1-Desoxy-1-piperidino-D-fructose. Das zweite Produkt, vermutlich das erwartete durch Ketosylamin-Umlagerung gebildete Derivat des Glucosamins, erwies sich als recht empfindlich. Es zersetzte sich beim Stehen und konnte nicht in reiner Form erhalten werden. Bei der Umsetzung von Fructose mit Piperidin erfolgt demnach bevorzugt eine Isomerisierung zwischen C-Atom 2 und 1 zu Glucose, die dann unter Amadori-Umlagerung weiterreagiert. Die Bildung von Glucosamin-Derivaten über eine Ketosylamin-Umlagerung erfolgt nur in untergeordnetem Masse.

Bei der Umsetzung von Fructose mit Piperidin konnten wir als Nebenprodukte zwei Reductone erhalten: Ein im Hochvakuum sublimierbares, extrem hygroskopisches zersetzliches Reducton und ein kristallisiertes stabiles Reducton, welches mit dem kürzlich von Weygand und Hodge[17] in seiner Struktur aufgeklärten Piperidinohexose-reducton (N-[1-Methyl-1,2,3-trihydroxy-cyclopenten(2)yliden-(4)]piperidiniumbetain) identisch ist. Die Bildung dieses Reductons dürfte demnach nicht aus Fructose direkt geschehen, sondern aus der durch primäre Umlagerung entstandenen Glucose über die 1-Desoxy-1-piperidino-D-fructose.

Wir haben ferner Fructose unter verschiedenen Reaktionsbedingungen mit

[14] K. Heyns, H. Paulsen, R. Eichstedt und M. Rolle, *Chem. Ber.* **90**, 2039 (1957).
[15] J. F. Carson, *J. Amer. Chem. Soc.* **77**, 1881, 5957 (1955); **78**, 3728 (1956).
[16] K. Heyns, R. Eichstedt und K. H. Meinecke, *Chem. Ber.* **88**, 1551 (1955).
[17] F. Weygand, H. Simon, W. Bitterlich, J. E. Hodge und B. E. Fisher, *Tetrahedron* **6**, 123 (1959).

Morpholin, Äthyl-n-butylamin, Di-n-butylamin, Dicyclohexylamin und Dibenzylamin behandelt, und konnten in keinem Fall Aminozucker als Umlagerungsprodukte nachweisen. In den Reaktionslösungen war Glucose, die bei der Umsetzung mit Piperidin das Hauptisomerisierungsprodukt darstellt, nur in geringer Menge vorhanden. Dagegen trat in allen Ansätzen D-Psicose als wesentliches Isomerisierungsprodukt der Fructose auf. Besonders bei der Umsetzung von Fructose mit Dicyclohexylamin und Morpholin waren etwa 20–25% der Fructose zu D-Psicose umgelagert.

Aus Ansätzen mit Dicyclohexylamin erhielten wir nach Abtrennung des Amins und Entfernung der überschüssigen Fructose durch Gärung mit Hefe einen stark angereicherten Sirup von D-Psicose, der nur geringe Mengen an Sorbose enthielt. Wir haben die D-Psicose in die gut kristallisierende Diisopropyliden-D-psicose überführt, welche gegenüber der von Steiger und Reichstein[18] beschriebenen entsprechenden Verbindung der L-Psicose den gleichen Schmelzpunkt und die gleiche Drehung in umgekehrter Richtung zeigt. Fructose erfährt demnach mit Dicyclohexylamin, Morpholin und anderen Aminen eine Isomerisierung zwischen C-Atom 2 und 3 zu D-Psicose. Das Verfahren kann zur einfachen Gewinnung von D-Psicose benutzt werden. D-Psicose ist neuerdings als Baustein des anti-bakteriell und anti-carcinogen wirksamen Antibiotikums 6-Amino-9-D-psicofuranopurins erkannt worden.[19]

Mit tertiären Aminen, wie Triäthylamin, in denen Fructose nur sehr wenig löslich ist, wird Fructose zu D-Psicose isomerisiert wie mit sekundären Aminen, jedoch in geringerem Ausmass . Schwach basische aromatische tertiäre Amine wie Pyridin sind auf Fructose und Sorbose ohne Einfluss.

EXPERIMENTELLER TEIL

D-Glucose-L-prolin (V). 64 g getrocknete D-Fructose und 7·4 g wasserfreies L-Prolin werden in 600 ml frisch absolutiertem Methanol unter Feuchtigkeitsausschluss bei 50° gelöst. Die Lösung wird 8 Std bei 60° gehalten. Es empfiehlt sich, die Reaktion papierchromatographisch zu verfolgen. Die Reaktion soll abgebrochen werden, wenn eine Maximalmenge an reduzierenden mit $AgNO_3$ anfärbbaren Hexose-Aminosäuren entstanden ist, aber die gleichzeitig fortschreitende Zersetzung des Prolins in Grenzen bleibt. Es sollen sich nicht mehr als 4 Ninhydrin-anfärbbare neue Aminosäuren gebildet haben, da sonst die spätere Trennung sehr schwierig ist. Wenn die Entwicklung der Chromatogramme erst abgewartet werden muss, kann die Reaktion durch Abkühlen auf −20° gestoppt und nach Auswertung der Chromatogramme durch Erwärmen fortgesetzt werden.

Das Methanol wird i. Vak. bei 40° entfernt und die Substanz auf eine Säule Lewatit S 100 H^+-Form (32 × 450 mm) aufgetragen und die überschüssige Fructose durch Waschen mit 2 l Wasser entfernt. Es wird mit 0·25 n Trichloressigsäure (abgekürzt TCE) eluiert und in 10 ml Fraktionen aufgefangen. Frakt. 48–260 enthalten reduzierende Stoffe und werden vereinigt. Sie enthalten alle gleichzeitig Ninhydrin-positive Stoffe und ab Frakt. 72 L-Prolin. Die TCE wird im Perforator mit Äther entfernt und die Lösung i. Vak. eingeengt. Zur völligen Entfernung der TCE mit Äther ist es zu empfehlen, die Perforation zu wiederholen, wenn die Lösung auf ein kleineres Volum eingeengt ist.

Zur weiteren Entfernung der Ninhydrin-positiven Substanzen wird die Substanz auf eine Säule Dowex 50 × 8 (200–400 mesh) H^+-Form (32 × 42 mm) gegeben, mit 300 ml Wasser gewaschen und mit 0·25 n TCE eluiert. Frakt. 50–76 enthalten alle reduzierende Substanz mit wenig Ninhydrin-positiven Aminosäuren. Sie werden vereinigt, die TCE mit Äther entfernt, i. Vak. eingeengt, ergibt 2·4 g Sirup. Dieser wird auf eine Säule Dowex 50 × 8 (200–400 mesh) H^+-Form (32 × 300 m) gegeben, mit 0·25 n TCE eluiert und in 10 ml Frakt. gesammelt. Frakt 33–52 enthalten reduzierende Substanz und sind frei von Ninhydrin-positiven Stoffen. Ein kleiner Vor- und Nachlauf mit Ninhydrin-positiven Substanzen wird verworfen. Frakt 36–41 werden vereinigt zu Frakt. A (800 mg

[18] M. Steiger und T. Reichstein, *Helv. Chim. Acta* **18**, 790 (1935).
[19] T. E. Eble, H. Hoeksema und G. A. Boyak, *J. Amer. Chem. Soc.* **81**, 1767 (1959).

Sirup), Frakt 42–48 ergeben Frakt B (620 mg Sirup, jeweils nach Abtrennen der TCE mit Äther und Einengen).

Die papierchromatographische Untersuchung der Trennungen im Fliessmittel Bu-Ä-W ergibt, dass vom sauren Austauscher die Zückeraminosäuren in folgender Reihenfolge eluiert werden: D-Mannose-L-Prolin, D-Fructose-L-Prolin, D-Glucose-L-Prolin. Jedoch treten von allen Substanzen weite Überlappungszonen auf, so dass eine mehrfache Säulentrennung erforderlich ist, s. Abb. 1.

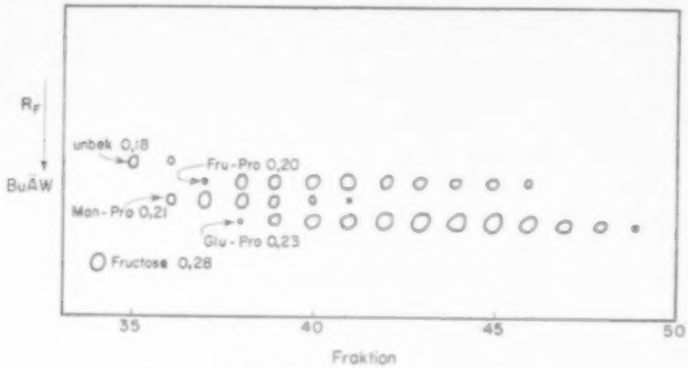

ABB. 1. Es ergibt sich folgendes Trennungsschema

Frakt. A	A_1 enthält 130 mg ----------	Frakt. D. enth. Man.-Prol.
	A_2 enthält 250 mg liefert 80 mg Krist. Glu-Prol.	
	A_3 enthält 170 mg liefert 80 mg Krist. Glu-Prol.	Frakt. C: C_1 180 mg liefert 80 mg Krist Fru.-Prol; C_2 70 mg
	A_4 enthält 70 mg ----------	
Frakt. B	B_1 enthält 90 mg liefert 30 mg Krist. Glu-Prol	
	B_2 enthält 100 mg liefert 100 mg Krist. Glu-Prol	
	B_3 enthält 60 mg liefert 30 mg Krist. Glu-Prol	

Frakt A wird auf eine Säule Dowex 50 × 8 (200–400 mesh) H^+-Form (18 × 460 mm) gegeben, mit 0·2 n TCE eluiert und nach den Papierchromatogrammen der Einzelfraktionen in 4 Fraktionen A_1 bis A_4 zusammengefasst, von denen die ersten beiden Mannose-, Fructose- und Glucose-Prolin, die letzten beiden Fructose- und Glucose-Prolin enthalten. Frakt B wird gleichfalls an Dowex 50 × 8 (200–400 mesh) H^+-Form (18 × 180 mm) mit 0·2 n TCE fraktioniert. Es werden 3 Fraktionen B_1 bis B_3 gebildet, die wechselnde Mengen Fructose- und Glucose-Prolin enthalten.

Die reinste Fraktion B_2 wird nach Abtrennen der TCE und Einengen zum möglichst trockenen Sirup in Methanol aufgenommen und mit absolutem Äther umgefällt. Nach Wiederaufnehmen in Methanol kristallisiert D-Glucose-L-Prolin aus. Die Reinigung durch Umfällen mit Methanol-Äther wird gleichfalls mit den anderen Fraktionen durchgeführt und diese mit den erhaltenen Kristallen angeimpft. Es werden insgesamt 320 mg reine krist. Substanz erhalten. Umkristallisieren erfolgt durch Lösen in 40 ml warmen Methanol mit einigen Tropfen Wasser, Einengen dieser Lösung auf die Hälfte und Kristallisieren im Eisschrank. Die Mutterlauge der Kristalle wird mit 40 ml Methanol verdünnt und die Lösung auf 20 ml eingeengt, worauf im Eisschrank unter Wiederholung dieser Operation weitere Fraktionen zu gewinnen sind.

Ausb. 300 mg. F 171°. Zers.

$[\alpha]_D^{20}$ −23° in 0 Min. (extrapoliert), −15° in 15 Min., +2° Endwert (c = 2 in H_2O)

$[\alpha]_D^{20}$ 0° (c = 1·5 in 2 n HCl) ($C_{11}H_{19}NO_7$ (277·3) Ber. C, 47·64; H, 6·91; N, 5·05; Gef. C, 46·63; H, 7·06; N, 5·16%). R_F 0·15 (BuEW); R_F 0·23 (BuÄW); $R_{Fructose}$ 0·73 (BuEW); $R_{Fructose}$ 0·82 (BuÄW).

Die vereinigten Mutterlaugen der Fraktionen A_3, A_4 B_1 und B_2 werden über eine Säule Dowex 50 × 8 (200–400 mesh) H^+-Form (12 × 290 mm) mit 0·2 n TCE fraktioniert. Aus der Hauptfraktion kristallisierte nach Beimpfung 80 mg D-Fructose-L-Prolin (IX), welches mit der aus Glucose und L-Prolin dargestellten Verbindung identisch ist.

Die Frakt. A_1 und A_2 werden zur Gewinnung von D-Mannose-L-Prolin (VI) noch zweimal an kleinen Säulen von Dowex 50 × 8 (200–400 mesh) H^+-Form (12 × 250 mm) mit 0·2 n TCE fraktioniert. Reines D-Mannose-L-prolin R_F 0·21 (BuÄW) lässt sich dabei nicht erhalten. Eine Reihe Fraktionen in denen diese Verbindung am meisten angereichert ist, wird nach Abtrennung der TCE zur

Trockne eingeengt, ausgewogen und spez. Drehung bestimmt. Diese beträgt zwischen $[\alpha]_D^{20}$ −12° bis −24°. Damit ist erwiesen, dass diese Verbindung die Manno-, die kristallisiert erhaltene dagegen, die Gluco-Konfiguration besitzt.

D-*Fructose*-L-*Prolin* (IX). 102 g getrocknete D-Fructose und 9·0 g getrocknetes L-Prolin werden in 1250 ml abs. Methanol unter Feuchtigkeitsausschluss bei 50° gelöst und 2·5 Std. bei 60° gehalten. Das Methanol wird i. Vak. abgedampft, der Sirup in Wasser gelöst und auf eine Säule Dowex 50 × 8 (200–400 mesh) H^+-Form (32 × 500 mm) gebracht, mit 1·5 l Wasser gewaschen, um die Fructose zu entfernen, und mit 0·2 n TCE eluiert in Fraktionen zu 10 ml. Frakt 2–137 enthalten D-Fructose-L-Prolin, ab Frakt 138 erscheint Prolin. Die vereinigten Fraktionen werden zur Entfernung von TCE mit Äther perforiert. Die Extraktion wird nach Einengen i.V. auf ein kleines Volum wiederholt. Der erhaltene Sirup wird mehrmals mit Methanol aufgenommen und eingeengt. Bei Wiederaufnahme in 100 ml Methanol kristallisieren in einigen Tagen 10·8 g D-Glucose-L-Prolin im Eisschrank. Durch Einengen der Mutterlauge und Aufnehmen in Methanol werden weitere 1·25 g in zwei Fraktionen erhalten.

Gesamtausb. 12·05 g (50% d.Th.).

Zum Umkristallisieren werden 4·0 g Rohprodukt in 600 ml Methanol heiss gelöst. Im Eisschrank und nach Einengen scheiden sich 2·7 g Kristalle ab, die 1 Mol. Kristall-Methanol enthalten. F 80–81°. $[\alpha]_D^{20}$ −82° (c = 3 in H_2O); $[\alpha]_D^{20}$ −87° (c = 2 in 2 n HCl) ($C_{11}H_{12}NO_7 \cdot CH_3OH$ (309·3) Ber. C, 46·60; H, 7·49; N, 4·53; Gef. C, 46·71; H, 7·41; N, 4·70%).

Werden 5·4 g des Rohproduktes in 100 ml Methanol mit 5% Wasser heiss gelöst, so kristallisieren in einigen Tagen 3·05 g aus. Diese Verbindung enthält 1 Mol Kristallwasser F 119° (Zers. bei 134°). $[\alpha]_D^{20}$ −86° (c = 2 in H_2O); $[\alpha]_D^{20}$ −89° (c = 2 in 2n HCl) ($C_{11}H_{12}NO_7 \cdot H_2O$ (295·3) Ber. C, 44·71; H, 7·17; N, 4·74; Gef. C, 44·80; H, 7·01; N, 4·54%).

Die Kristall-Methanol-haltige Verbindung gibt bei 0·1 Torr 80° über P_2O_5 getrocknet den Methanol ab. Das Kristallwasser kann nicht ohne Zersetzung der Verbindung abgetrennt werden. Freie Verb. F 137° (Zers). $[\alpha]_D^{20}$ −92° (c = 2 in H_2O) ($C_{11}H_{12}NO_7$ (277·3) Ber. C, 47·64; H, 6·91; N, 5·05; Gef. C, 46·84; H, 6·82; N, 5·14%).

R_P 0·12 (BuEW); R_P 0·20 (BuÄW); $R_{Fructose}$ 0·61 (BuEW); $R_{Fructose}$ 0·71 (BuÄW).

L-*Sorbose*-L-*Prolin* (XI). 90 g getrocknete L-Sorbose und 8·2 g wasserfreies L-Prolin werden in 500 ml wasserfreiem frisch destillierten Dimethylsulfoxyd suspendiert und die Lösung 3 Tage bei 60° gehalten. Das Dimethylsulfoxyd wird bei 0·2 Torr bei einer Temp. unterhalb 50° abdestilliert. Der Rückstand wird mehrmals in Wasser aufgenommen und wieder im Hochvakuum eingeengt, um das Dimethylsulfoxyd weitgehend zu entfernen. Der erhaltene Sirup wird in Wasser gelöst, auf eine Säule Lewatit S 100 H^+-Form (32 × 540 mm) gegeben, die überschüssige Sorbose mit 1·5 l Wasser ausgewaschen und die Hexose-Aminosäuren mit 0·2 n TCE eluiert, wobei Fraktionen zu 10 ml aufgefangen werden. Frakt 65–240 enthält reduzierende Substanz und wird vereinigt. Ab Frakt 170 erscheint L-Prolin. Die vereinigten Fraktionen werden zur Entfernung der TCE im Perforator mit Äther extrahiert, i. Vak. eingeengt und auf eine Säule Dowex 50 × 8 (200–400 mesh) H^+-Form (25 × 320 mm) gebracht und mit 0·25 n TCE eluiert und 10 ml-Fraktionen aufgefangen. Frakt 14–336 enthalten alle reduzierende Substanzen, sie sind frei von Prolin. Nach Entfernen der TCE durch Perforieren mit Äther und Einengen i. Vak. werden 3·9 g Sirup erhalten. Dieser wird in wenig abs. Methanol gelöst und die Substanz mit abs. Äther gefällt. Nach Wiederaufnahme der Substanz in abs. Methanol scheiden sich beim Stehen Kristalle ab, durch Einengen der Mutterlauge und Stehen im Eisschrank ergibt sich eine zweite Fraktion. Ausb. 1·8 g, 26% d.Th.

Das L-Sorbose-L-Prolin wird in 100 ml heissem Methanol mit einigen Tropfen Wasser gelöst. Kristalle im Eisschrank. Durch Einengen der Mutterlauge auf die Hälfte und Zugabe von 100 ml Methanol ergibt sich eine weitere Fraktion (Ausb. 1·6 g) F 167° (Zers.) $[\alpha]_D^{20}$ −52° (c = 2 in H_2O keine Mutarotation).

($C_{11}H_{19}NO_7$ (277·3) Ber. C, 47·64; H, 6·91; N, 5·05; Gef. C, 47·04; H, 6·65; N, 5·13%).

R_P 0·07 (BuEW); $R_{Sorbose}$ 0·56 (BuEW).

Die Mutterlauge wird eingeengt und mit Äther eine amorphe Substanz gefällt $[\alpha]_D^{20}$ −15°. Diese enthält zu 3/4 L-Sorbose-L-Prolin und zu 1/4 eine Aldose-L-prolin-Verbindung (vermutlich L-Gulose-L-prolin). R_P 0·08 (BuEW) $R_{Sorbose}$ 0·64 (BuEW).

Fructose mit Piperidin. 7·0 g getrocknete Fructose wird in 45 ml dest. Piperidin gelöst und 8 Std. auf 60° gehalten. Papierchromatographisch ist im Fliessmittel P-A-W ein N-Glycosid nachweisbar. Das Piperidin wird schonend i. Vak. weitgehend abdestilliert. Der Sirup wird in 40 ml Methanol

gelöst und 2·5 ml Eisessig als Umlagerungskatalysator zugefügt. Es wird i. Vak. eingeengt und die Substanz auf eine Säule Lewatit S 100 H^+-Form (32 × 480 mm) gegeben. Zur Entfernung des Zuckers wird mit 2 l Wasser gewaschen. Im Durchlauf befindet sich Fructose und Glucose. Es wird eluiert mit 0·25 n HCl. Alle Silbernitrat-positiven Fraktionen werden vereinigt und i. Vak. unterhalb 45° eingeengt. Um die Salzsäure weitgehend zu entfernen, wird das Einengen nach Wasserzugabe mehrmals wiederholt. Die Substanz wird zur weiteren Reinigung auf eine Säule Dowex 50 × 8 (200–400 mesh) H^+-Form (32 × 440 mm) gebracht und mit 0·25 n HCl eluiert und in 4 ml Fraktionen aufgefangen. Die ersten Frakt 203–223 enthalten das reine Hauptprodukt von R_F 0·28 (Bu-E-W). Sie werden eingeengt und es ergibt sich eine sirupöse Substanz, die in allen Eigenschaften (Papierchromatographie, Reduktionseigenschaften, Drehung) mit dem Hydrochlorid der 1-Desoxy-1-piperidino-D-fructose von Hodge und Rist[8] übereinstimmt. Die letzten Frakt. 276–309 enthalten zu 75% angereichert das zweite Umlagerungsprodukt, vermutlich ein entsprechend N-substituiertes Glucosamin R_F 0·34 (Bu-E-W). Es gelingt nicht, dieses Produkt in reiner Form zu gewinnen. Beim Stehen zersetzt es sich unter Bildung eines Produktes vom R_F 0·23 (Bu-E-W).

Ein anderer Ansatz von Fructose mit Piperidin wird 20 Std. bei 60° stehen gelassen. Das Piperidin wird bei 0·2 Torr abdestilliert und nach Aufnahme mit Methanol wieder eingeengt. Bei 0·1 Torr und 40° unter N_2-Atmosphäre sublimiert ein Reducton ab. Die Nadeln zerfliessen sofort bei Luftzutritt und die empfindliche Substanz färbt sich dunkel. Der sirupöse Rückstand wird in Methanol aufgenommen, dabei scheiden sich sofort Kristalle ab F 230°. Die Substanz ist identisch mit dem Piperidino-hexose-reducton von Hodge[8].

D-*Psicose*. 18·1 g getrocknete D-Fructose werden in 36·3 ml frisch destilliertem Dicyclohexylamin und 130 ml abs. Methanol gelöst und unter Rühren 20 Std. bei 60° gehalten. Die Lösung wird i. Vak. unterhalb 45° eingeengt, der Sirup mit 200 ml Wasser versetzt. Die sich abscheidende Dicyclohexylamin-Phase wurd durch dreimaliges Ausschütteln mit Äther entfernt. Die Lösung wird über eine Säule Lewatit S 100 H^+-Form (32 × 460 mm) geschickt und der Durchlauf i. Vak. zum Sirup eingeengt. Zur restlosen Entfernung von Methanol wird nochmals mit Wasser eingeengt, ergibt 17·8 g Sirup. Dieser wird in 250 ml Wasser gelöst, mit $(NH_4)_2CO_3$ auf p_H 7·5 gebracht, 300 mg $NH_4H_2PO_4$ und 2 g Bäckerhefe zugegeben und 3 Tage bei 37° bebrütet. Die Hefe wird mit wenig Kohle abzentrifugiert, die Lösung durch einen Bakterienfilter filtriert, i. Vak. zum Sirup eingeengt und zur möglichst vollständigen Entfernung des Wasser mehrmals mit Methanol abgedampft. 7·4 g Sirup mit einem Gehalt von etwa 80% D-Psicose $[\alpha]_D^{20}$ +2 (c = 3 in H_2O) R_F 0·26, $R_{Fructose}$ 1·32 (Bu-E-W), $R_{Fructose}$ 1·25 (P-A-W). Die Hauptverunreinigung ist eine Ketose vom R_F 0·16, $R_{Fructose}$ 0·81 (B-E-W), $R_{Fructose}$ 0·72 (P-A-W), welcher mit Sorbose übereinstimmt. 3 g des völlig getrockneten Sirups wird mit Aceton nach Steiger u. Reichstein[18] in die Diisopropyliden-D-psicose überführt. 210 mg Krist. aus Petroläther. F. 57°.

$[\alpha]_D^{20}$ −98 (c = 2 in Aceton) ($C_{12}H_{20}O_6$ (260·3) Ber. C, 55·37; H, 7·75; Gef. C, 55·89; H, 7·79%)

Papierchromatographie. Es werden folgende Gemische verwendet: n-Butanol-Eisessig-Wasser (70:7:23) = Bu-E-W; Pyridin-Isoamylalkohol-Wasser (7:7:6) = P-A-W; sek-Butanol-Äthylmethylketon-Wasser (25:4:16) = Bu-Ä-W, in dem allein die isomeren Hexose-prolin-Verb. zu trennen sind. Papier Schleicher-Schüll 602 h:p. Anfärben erfolgt mit ammoniakalischer $AgNO_3$-Lösung, Ninhydrin und Harnstoff-Phosphorsäure für Ketosen. P-A-W-Chromatogramme müssen vor dem Besprühen mit ammon. $AgNO_3$ erst mit Essigsäure besprüht werden.

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FACTORS AFFECTING STABILITY AND EQUILIBRIA OF FREE RADICALS

STERIC FACTORS IN HYDRAZYLS

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Abstract—The preparation of 1,1-diphenyl-2-(2,4- and 2,6-dinitrophenylhydrazine) is described. Lead peroxide converts them into hydrazyl free radicals, but only the latter may be isolated in crystalline form. Ultra-violet and visible absorption spectra of hydrazines and hydrazyls are compared.

Factors Affecting the Stability of Equilibrium Free Radicals

IT is today well established that the stability of triarylmethyl free radicals is due both to *conjugative* and to *steric factors*.[1,2] Changes in dissociation of *para*-substituted hexa-arylethanes are usually attributed to the former factors, while the latter are reflected in the large effects of *ortho*-substituents on the same dissociation.

The *conjugative factors* were the first to be explored, and it was found that both electron-repelling and electron-attracting substituents enhance the dissociation of hexa-arylethanes.[1b,2b] In the nitrogen series, Wieland observed[3] that electron-repelling substituents (Me_2N, MeO, Me) increase the dissociation of tetra-arylhydrazines while electron-attracting groups (Br, NO_2) exert the opposite effect. This difference between carbon and nitrogen radicals was tentatively explained[4] and confirmed by newer measurements on the dissociation of 1,1,4,4,-tetra-aryl-2,3-benzoyltetrazanes.[5]

The steric factors may be further subdivided into two antagonistic steric effects. The *direct steric effect** is exerted on the central bond in the dimer (hexa-arylethane, tetra-arylhydrazine, diarylperoxide); owing to steric repulsion this bond is lengthened and weakened, favouring the dissociation. The *indirects teric effect** is exerted on the aryl groups in the radical: steric repulsions causes them to be twisted, the radical

* In order to carry the subsequent discussion we had to find names for these two steric effects: the first is purely steric, the second is exerted through reduction of conjugation, therefore the names "direct-indirect" were adopted.

[1] [a] W. A. Waters, *The Chemistry of Free Radicals*. Butterworths, London (1948); [b] J. E. Leffler, *The Reactive Intermediates of Organic Chemistry* Chapters 1 and 4. Interscience, New York (1956); [c] C. Walling, *Free Free Radicals in Solution* pp. 162, 524, 530. John Wiley, New York (1957); [d] D. J. E. Ingram, *Free Radicals as Studied by Electron Spin Resonance* p. 138. Butterworths, London (1958); [e] D. H. Hey, *Ann. Rep.* **37**, 250 (1940).

[2] [a] A. E. Remick, *Electronic Interpretations of Organic Chemistry* (2nd Ed) p. 326. John Wiley, New York (1949); [b] M. J. S. Dewar, *The Electronic Theory of Organic Chemistry* pp. 382–393. John Wiley, New York (1955); (1950); [c] G. W. Wheland, *Resonance in Organic Chemistry* pp. 382–393. John Wiley, New York (1955); [d] J. Hine, *Physical Organic Chemistry* Chap. 18. McGraw-Hill, New York (1956); [e] O. A. Reutov, *Theoreticheskie problemy organicheskoi khimii* (Edited by Moscow Univ.) Chap. 5 (1956); [f] G. E. K. Branch and M. Calvin, *The Theory of Organic Chemistry* p. 326. Prentice-Hall, New York (1941).

[3] H. Wieland, *Die Hydrazine* p. 71. F. Enke Verlag Stuttgart (1913); *Liebigs Ann.* **381**, 200 (1911); *Ber. Dtsch. Chem. Ges.* **48**, 1078 (1915); H. Wieland and A. Wecker, *Ibid.* **55**, 1804 (1922), and further references therein.

[4] H. Burton and C. K. Ingold, *Proc. Leeds Phil. Soc.* **1**, 430 (1929); G. N. Lewis and D. Lipkin, *J. Amer. Chem. Soc.* **63**, 3232 (1941); M. J. S. Dewar, *Ibid.* **74**, 3353 (1952).

[5] N. Schwartz and W. K. Willmarth, *J. Chem. Phys.* **20**, 748 (1952); W. K. Willmarth and N. Schwartz, *J. Amer. Chem. Soc.* **77**, 4543, 4551 (1955).

assumes a non-planar form ("propeller" for triarylmethyls) which reduces the conjugation in the radical and thus the dissociation is reduced. These steric effects can hardly be separated, but the former usually predominates over the latter, the net result being that bulky *ortho*-alkyl groups tremendously stabilize triarylmethyl and even diarylmethyl radicals[6] (the effect is largely steric since *o*-methoxy groups do not show it[7]). Only in some bridged compounds with fixed steric configuration can these two effects be separated, e.g. the tripticyl radical is instable[8] because only the indirect steric effect is at work (for a stable triphenylmethyl radical with rigid oxygen bridges *cf.*[9]) In the case of oxygen free radicals, bulky t-butyl[10] or phenyl[11] groups in both *ortho* positions are essential for the stability of aroxyls; here only the direct steric effect is involved.

Nitrogen free radicals differ from carbon free radicals in that they do not react with oxygen. Indeed, this is one of the reasons why 1,1-diphenyl-2-picryl-hydrazyl[12] (2,4,6-yl*: I, R = R′ = H) is so widely used as a standard free radical for electron spin resonance measurements.[1d,13] The other reason is its stability: it has no tendency towards dimerization in solution or in crystalline state even at low temepratures[14] and it does not disproportionate even on prolonged heating. The unreactivity of 2,4,6-yl extends even to the reaction with the characteristic reagent for nitrogen radicals, NO. On the other hand 2,4,6-yl readily gives hydrogen abstractions[15] and other redox reactions[16] and reacts with stable[12] or transient[17] carbon free radicals; these are its

* This shorthand notation will be preferred over the usual one, DPPH, because it may be applied to hydrazines and hydrazyls differently substituted with nitro-groups.

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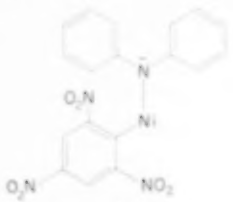
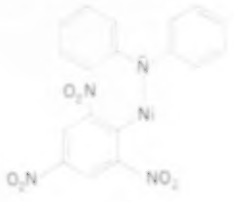
two main chemical applications (oxidizing action in redox systems, and radical scavenging and counting; for limitations in the latter reaction see[18] and for chemical dosimetry of ionising radiations see[19]).

2,4,6-Yl is exceptional among other nitrogen[3] or hydrazyl[20] free radicals, not only because of its amazing stability, but, and especially, because of the contradictory effect of nitro groups, which in 2,4,6-yl contrary to all other nitrogen free radicals, exert a stabilizing influence. This "puzzling"[1b] contradiction disappears if one postulates that the stability of 2,4,6-yl is not electronically, but sterically conditioned. Until now no consistent discussion of the steric effects on the stability of nitrogen free radicals has been published, though for carbon and oxygen free radicals the importance of the steric factor has clearly been recognized.[1,2] Only an indirect steric effect has been mentioned[21] in the case of tetra-arylhydrazines. We suppose on the contrary, that in 2,4,6-yl a stabilizing direct steric effect is operating.

The studies of free radicals related with 2,4,6-yl did not bear on the sterical but on the electronic effects: related radicals were prepared, modifying the diarylamino moiety through various substituents R and R′ in I.[22–25] Even in the two somewhat



I



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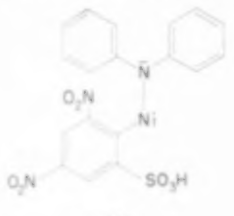
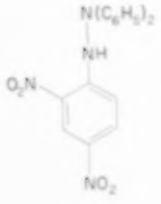


III

differently related radicals II[26,27] and III,[26] the steric relationships are practically unaltered.

We therefore investigated hydrazyl free radicals with modified steric environments.



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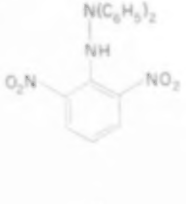
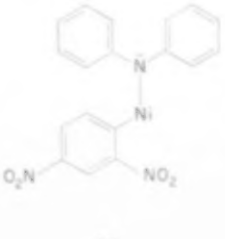
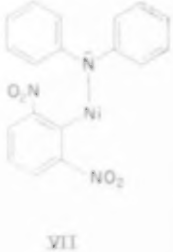


V



VI



VII

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[25] R. G. Bennett and A. Henglein, *J. Chem. Phys.* **30**, 1117 (1959).
[26] H. S. Jarrett, *J. Chem. Phys.* **21**, 761 (1953).
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The present paper describes the synthesis and properties of radicals VI and VII in which the picryl group in 2,4,6-yl has been replaced by a 2,6- or 2,4-dinitrophenyl group.

RESULTS AND DISCUSSION

The general method of Goldschmidt and Renn[12] was followed, namely the nucleophilic aromatic substitution through 1,1-diphenyl-hydrazine of a halogen atom activated by *o*- and *p*-nitro groups, to yield the triarylhydrazines IV and V respectively, which were subsequently dehydrogenated with lead peroxide (PbO_2). While 2,6-dinitrochlorobenzene reacted normally, though much less readily than picryl chloride, it was not possible to prepare 2,4-ine (IV) starting from 2,4-dinitrochlorobenzene which yielded only solvolysis products. However, 2,4-ine was readily prepared with the much more reactive 2,4-dinitrofluorobenzene, which reacted with 1,1-diphenylhydrazine almost as rapidly as picryl chloride. On the other hand, 1,1-diphenylhydrazine did not substitute *p*-nitrofluorobenzene even under drastic conditions (had this reaction been successful, a third radical with both *ortho* positions free from steric interference would have been prepared).

The oxidation with lead peroxide proceeded normally, yielding in both cases violet solutions (in chloroform, 1,2-dichloroethane or benzene), very similar with the 2,4,6-yl solutions. The radical 2,6-yl (VII) was easily isolated through evaporation of the solvent and recrystallization from benzene–ligroin.

However, the radical 2,4-yl (VI) could not be isolated in crystalline state, because its solutions became brown on concentration (even at low temperature and in the absence of air), and its benzenic solution prepared in the cold deposited only a brown product on dilution with petroleum ether. This brown product gave no paramagnetic resonance absorption and consequently was no longer a radical. In dilute solution, 2,4-yl is stable for several days.

This difference between 2,4-yl and 2,6-yl strikingly illustrates the importance of steric effects for the stability of triarylhydrazyl free radicals: while in both 2,4-yl and 2,6-yl the electronic effects are comparable, only 2,6-yl with both *o*-positions occupied by bulky nitro-groups is stable and monomeric in crystalline state; with one *o*-position free, 2,4-yl is appreciably less sterically hindered than 2,4,6-yl or 2,6-yl and that explains why it is not able to be isolated in solid state; whether the brown compound is formed from it through dimerization, disproportionation, or other reaction such as intramolecular substitution to a phenazone derivative cannot be definitely ascertained as yet. Though it is not safe to draw conclusions from unsuccessful experiments, we believe that the failure to isolate 2,4-yl in solid state is significant, and that it proves that 2,4-yl is less stable than 2,6-yl.

Absorption spectra

Ultra-violet and visible absorption spectra were determined for the three hydrazines 2,4,6-ine, 2,4-ine (IV) and 2,6-ine (V) in neutral and alkaline methanol solutions, and for the three radicals 2,4,6-yl, 2,4-yl and 2,6-yl in 1,2-dichloroethane solution. The absorption spectra are given in Figs. 1–3, and through graphical interpolation the bands shown in Table 1 were found. The results for 2,4,6-yl and 2,4,6-ine are in agreement with reported spectra[28,29] (no data below 260 mμ were previously given).

[28] S. Goldschimdt and F. Graef, *Ber. Dtsch. Chem. Ges.* **61**, 1858 (1928).
[29] R. H. Poirier, E. J. Kahler and F. Benington, *J. Org. Chem.* **17**, 1437 (1953).

The relevant features are summarized in the following items:

1. All three hydrazines have three bands in neutral solution, at ca. 225, 265 and 330 mμ (the last band is shifted with 60 mμ towards the visible and its intensity is decreased in 2,6-ine). The total Σf value is remarkably constant (1·1). for all three hydrazines.

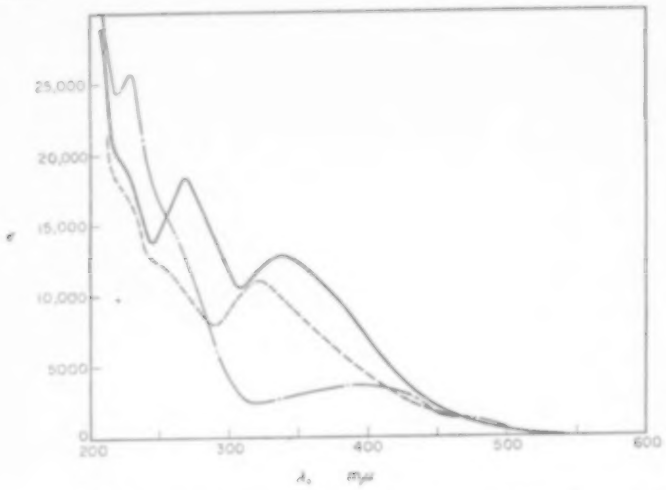


FIG. 1. Absorption spectra of 2,4-ine (full line), 2,6-ine (dashed–dotted line) and 2,4,6-ine (dashed line) in methanol.

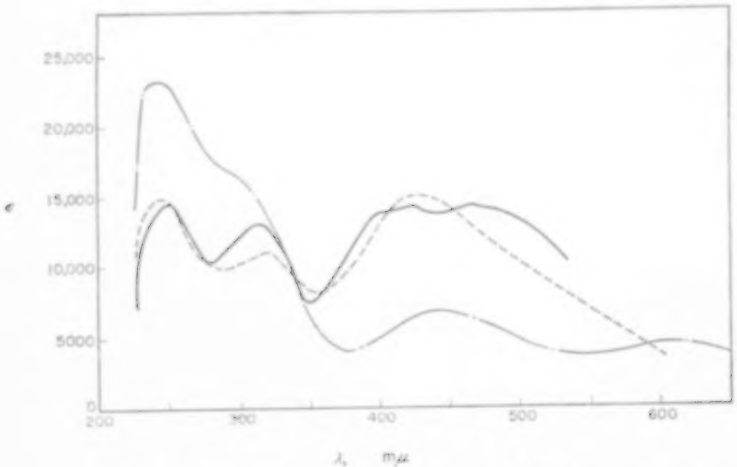


FIG. 2. Absorption spectra of 2,4-ine (full line), 2,6-ine (dashed–dotted line) and 2,4,6-ine (dashed line) in 90% methanol with 0·5 N sodium hydroxide.

2. In basic solution the situation is less clear-cut: 0·05 N NaOH solutions considerably affect the colour of 2,4,6-ine, yielding a spectrum essentially identical with that of 0·5 N NaOH solutions; however, 2,4-ine and even more 2,6-ine do not change colour in 0·05 N NaOH solutions and the absorption spectra do not differ from spectra determined in neutral solution. The greater acidity of 2,4,6-ine is not surprising, since

it has one activating nitro group more. In 0·5 N NaOH solution, 2,4-ine becomes violet-brown like 2,4,6-ine, and 2,6-ine becomes green. The spectrum of 2,4,6-ine presents three bands at ca. 250, 320 and 430 mμ; the last band is split into two closely situated bands in 2,4-ine (ca. 400 and 430 mμ: interpolation errors were rather high in this case owing to the shape of the band envelope) and into two distanced bands

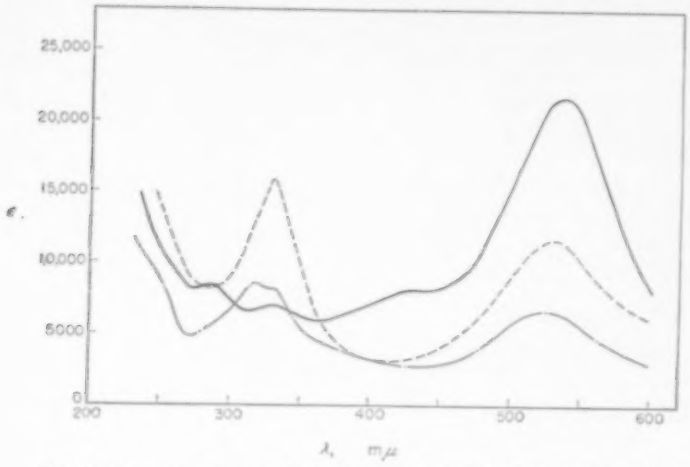


FIG. 3. Absorption spectra of 2,4-yl (full line), 2,6-yl (dashed–dotted line) and 2,4,6-yl (dashed line) in 1,2-dichloroethane.

in 2,6-ine (440 and 610 mμ). As in the previous case, the longest wave length band in 2,6-ine has a considerable lower frequency and intensity than in the other two hydrazines, and the three Σf values are fairly constant (1·5). The shape of the spectrum for 2,4,6-ine in basic medium suggests the possible presence of a small band completely masked, at about 290 mμ with ε ca. 4500.

3. All three free radicals present a band at 530 mμ, responsible for the violet colour, though its wave length and intensity in 2,6-yl are smaller than in the other two radicals (this is the reason that solutions of 2,6-yl have nearly the same nuance but are more pale than solutions in equal concentrations of the other two radicals), and another band at 330 mμ. However, the remainder of the spectrum is quite different: 2,4,6-yl contains no more bands; 2,6-yl has a band at 315 mμ and possibly a completely masked band with λ_{max} 375 mμ, ε_{max} 2800, f 0·048, whose presence is suggested by the asymmetry of the descending branches of the 330 mμ band; and 2,4-yl contains two distinct bands at 288 and 435 mμ. The last spectrum was determined immediately after oxidation with PbO_2 of a solution of 2,4-ine in dichloroethane; the possibility is however not excluded that in this case the decomposition products of the radical manifest themselves in the absorption spectrum: the 435 mμ band could possibly originate in this way, and if this were true the sensibly higher Σf value for 2,4-yl would come into line with the other two radicals (0·5).

Previous spectra of 2,4,6-yl were determined in chloroform [28,29](cf. also[22,30]). Using 1,2-dichloroethane we advanced about 30 mμ into the ultra-violet range. In order to enlarge still further the spectral range, the spectra of 2,6-yl and 2,4,6-yl were also determined in di-n-butyl ether (the solubility is satisfactory) and in cyclohexane

[28] R. H. Poirier and F. Benington, *J. Org. Chem.* **19**, 1847 (1954).

TABLE 1. ABSORPTION BANDS OF 1,1-DIPHENYL-2-POLYNITROPHENYL-HYDRAZINES AND -HYDRAZYLS[a]

Compound and solvent	2,4-Dinitro						2,6-Dinitro						2,4,6-Trinitro					
	Apparent[b]		Interpolated[c]				Apparent		Interpolated				Apparent		Interpolated			
	λ	ε	λ	ε	f	Σf	λ	ε	λ	ε	f	Σf	λ	ε	λ	ε	f	Σf
Hydrazine Methanol	225*s*	19000	231	12000	0·195	1·17	230	25800	230	25000	0·47	1·02	215*s*	17700	222	17000	0·51	1·18
	267	18800	267	14000	0·37		260*s*	15000	260	13000	0·40		260*s*	11800	257	7500	0·163	
	338	12800	338	12800	0·61		394	3700	394	3700	0·151		322	11000	322	11000	0·51	
Hydrazine (sodium salt) 0·5 N NaOH in 90% methanol	250	14800	250	14500	0·62	1·65	245	23000	242	23000	0·95	1·62	244	15200	244	15000	0·62	1·40
	310	13000	310	13000	0·40		300*s*	16000	305	16000	0·42		320	11100	320	8000	0·125	
	425	14200	395	13000	0·41		440	6800	440	6800	0·153		431	15100	431	15100	0·65	
	465	14300	490	13500	0·22		610	4400	610	4400	0·101							
Hydrazyl	288	8400	286	6300	0·132	0·91	315	8400	313	7500	0·16	0·49						0·59
	330	7000	332	7000	0·25		330*s*	8300	332	7000	0·20		332	15800	332	15800	0·37	
	435[d]	8000	436	7500	0·16													
1,2-Dichloroethane	537	21700	536	21700	0·37		513	6700	513	6700	0·13		530	11700	530	11700	0·22	

[a] Wavelengths in mμ; spectral limits were 210 mμ for methanol and 240 mμ for dichloroethane.

[b] A shoulder (inflexion) is denoted by *s*.

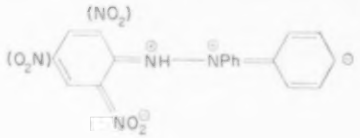
[c] The interpolation was done graphically; in view of the considerable uncertainties in some interpolated data for λ_{max} and ε_{max}, f values were not calculated through integration, but through the approximate formula $f = 4{\cdot}32 \cdot 10^{-9} \cdot \varepsilon_{max} \cdot \Delta\nu_{max}$}. Completely masked bands for basic 2,4,6-ine and 2,6-yl, apparent only in the interpolation procedure, are not separately shown (see text).

[d] This band is possibly due to decomposition products of the radical (see text and experimental part).

(very small solubility). An extremely intense band at the spectral limit (205 mμ) was apparent for both radicals with a slight inflexion at 237 mμ. Small hipso- and hypochromic effects were noted in the solvent sequence dichloroethane → dibutyl ether → cyclohexane.

The assignment of electronic transitions can be done, tentatively and qualitatively, for all bands excepting those with shortest wave lengths.

The band with longest wave length in the hydrazine spectrum (neutral solution) is due to the partial chromophore[31,32] comprising the polynitrophenyl group and the adjacent NH group. A conjugation throughout the molecule would cause absorption at wave lengths greater than 400 mμ. Such a conjugation is impossible both for electronic (because it would imply highly improbable structures like VIII with adjacent positive nitrogen atoms) and for steric reasons. Molecular models after Stewart-Briegleb show that diphenylamine is non-planar; in the arrangement nearest to



VIII

coplanarity the two phenyl rings make a dihedral angle of ca. 150° (this is the largest possible dihedral angle). In 1,1-diphenylhydrazine the same situation occurs, but it is apparent that the two hydrogens bonded to nitrogen are not equivalent: one of them is completely screened by the hydrogen atoms in *ortho*-positions. This N-bonded hydrogen is to be found also in triarylhydrazines, namely IV and V, since it cannot be replaced by other groups. The polynitroaryl group is perpendicular to the approximate plane of the two phenyl groups. Nitro groups in *ortho* positions have no space for free rotation and they are twisted out from conjugation with the aromatic ring. The situation must parallel the configuration of picryl iodide, where X-ray diffraction studies showed[33] that *ortho*-nitro groups were twisted and had large C—N distances, while the *para*-nitro group was coplanar and had a small C—N distance (an X-ray diffraction study of 2,4,6-ine, 2,4,6-yl and related substances would be highly interesting, not only because it would clear up the problem of steric configurations, but also because it would show how solvent molecules are complexed in radical crystals[34–36]).

While the lack of conjugation through two hydrazinic nitrogen atoms has been agreed upon,[37] it seems that even in diphenylamine derivatives and related substances, a conjugative effect is absent,[32] although previous reports reported the contrary.[37,38]

The second band in the spectra of hydrazines, at ca. 265 mμ, can possibly be due to the other partial chromophore, the diphenylamino moiety. The hipsochromic shift from the corresponding band in diphenylamine (285 mμ) could be ascribed to

[31] P. Grammaticakis, *Bull. Soc. Chim. Fr.* **99**, 1372 (1954) and previous papers.
[32] V. A. Izmail'ski and K. A. Nuridjanian, *Dokl. Akad. Nauk SSSR* **129**, 1053 (1959); **133**, 594 (1960).
[33] G. Hase and H. M. Powell, *J. Chem. Soc.* 1398 (1940).
[34] M. Sternberg, *C. R. Acad. Sci., Paris* **240**, 990 (1955).
[35] J. A. Lyons and W. F. Watson, *J. Polymer Sci.* **18**, 141 (1955).
[36] A. E. Arbuzov, F. G. Valitova, N. S. Garif'ianov and B. M. Kozyrev, *Dokl. Akad. Nauk SSSR* **126**, 774 (1959).
[37] F. Bohlmann, *Chem. Ber.* **84**, 490 (1951).
[38] H. H. Jaffé, *J. Chem. Phys.* **22**, 1430 (1954).

steric factors. (*Cf.* recent illustrations of absorption due to partial chromophores in tetraphenyls[39] and in pyrylium salts[40]).

In hydrazyl free radicals and in hydrazine anions, both electronic and steric factors are changed: electronically, structures like VIII with two positive adjacent nitrogens are no more involved; sterically, the configuration has changed owing to modified hydradization in the radical, and to disparition of the N—H . . . O—N hydrogen bond.[29,30] Thus, conjugation throughout the molecule is allowed, giving rise to the 530 mμ band in the radical and to absorption beyond 400 mμ in hydrazine anions.

The smaller number of bands in 2,4,6-yl and 2,4,6-ine anion indicates a higher degree of symmetry, as in the well-known case of malachite green and crystal violet. The splitting of the longer wave length band of 2,6-ine in basic medium (like 2,4-ine) and the exceptional bathochromic and hypochromic effects observed for the longer wave length bands in 2,6-ine in neutral and basic medium, can be accounted for by admitting that in 2,6-ine the two nitro groups are not equivalent, as formula V would suggest.

The band at 300–330 mμ in hydrazyls and hydrazine anions can analogously be ascribed to electronic transitions involving a semi-resp. fully-occupied orbital in the diphenylamino moiety.

Infra-red and ESR spectra will be reported later.

EXPERIMENTAL

It was claimed[41] that the preparation of 1,1-diphenylhydrazine was critical for the yield and the quality of 2,4,6-yl. Since our results are somewhat different, we are describing the preparation of 1,1-diphenylhydrazine, 2,4,6-ine and 2,4,6-yl.

Preparation of 1,1-*diphenylhydrazine*. Diphenylnitrosamine[42,43] (40 g, 0·2 moles), m.p. 66–67° (from ligroin) in 200 ml ethanol with 64 g (1 mole) high-grade zinc powder, was gradually treated with external cooling with 80 ml glacial acetic acid. The temp rising suddenly (30–40°), was kept between 20–25° by cooling and addition rate. The mixture was stirred for 1–2 hr longer until a sample failed to develop a blue colour on dilution with conc HCl. The white zinc complex was filtered off, then washed with ethanol (air moisture causes heating, and occasionally zinc acetate crystallizes in the filtrate), and the filtrate was treated with 2 l. HCl *d* 1·19. 1,1-Diphenylhydrazine hydrochloride was filtered off on sintered glass filter, washed with conc HCl and dried in a dessiccator (90–95% yield). The recrystallization[41] from HCl was accompanied by considerable losses and was unnecessary if the reduction has been carried out cautiously. If in the reduction anhydrous ethanol and acetic acid are used, the reaction proceeds less rapidly and less exothermally, the zinc powder does not become white and does not become warm on filtration, but the yield is substantially the same, an anhydrous medium is therefore unnecessary. By alkalinization, ether extraction and distillation under reduced pressure[41,43] 1,1-diphenylhydrazine is obtained as a colourless liquid with b.p. 165°/5 mm, b.p. 172°/8 mm, in 70–80% yield.

Preparation of 1,1-*diphenyl-2-picryl-hydrazine and -hydrazyl*. Picryl chloride was prepared from pyridine picrate with phosphorus oxychloride[44]* (which cannot be replaced by thionyl chloride). Equivalent amounts (20 mmoles) of diphenylhydrazine (3·7 g), picryl chloride (5·0 g) and sodium bicarbonate (1·7 g) in hot ethanol[29] yielded in 30 min the orange-red 2,4,6-ine (7·8 g, 99%), which may

* Compare.[45]

[29] R. H. Wiley and B. J. Wakefield, *J. Org. Chem.* **25**, 132 (1960).
[40] A. T. Balaban, V. E. Sahini and E. Keplinger, *Tetrahedron* **9**, 163 (1960); A. T. Balaban and C. D. Nenitzescu, *Izv. Akad. Nauk SSSR., Otdel khim. Nauk* 2064 (1960).
[41] A. E. Arbuzov and F. G. Valitova, *Zh. Obshchei Khim.* **27**, 2354 (1957).
[42] O. Witt, *Ber. Dtsch. Chem. Ges.* **8**, 855 (1875).
[43] E. Fischer, *Liebigs Ann.* **190**, 67, 174 (1878).
[44] H. Wieland and E. Müller, *Ber. Dtsch. Chem. Ges.* **46**, 3308 (1913).
[45] R. Boyer, E. V. Spencer and G. F. Wright, *Canad. J. Res.* **24B**, 200 (1946).
[46] K. Okón, *Rocz. Chem.* **33**, 45 (1959).

be recrystallized from benzene–ethanol or chloroform–ethanol, m.p. 174–175°. The conversion into 2,4,6-yl was effected by shaking for 2 hr with the equal amount of anhydrous sodium sulphate and a 20-times larger amount of lead peroxide in chloroform or benzene, and diluting after filtration with ether or ligroin. The radical crystallizes with solvent molecules which are lost by cautious heating under vacuum, and which caused the differences initially observed in the m.p. (*cf.*[34–36] and the last reference from[16]) and were responsible for variations in data concerning radical scavenging and ESR measurements.[36]

Preparation of 1,1-*diphenyl*-2-(2,4-*dinitrophenyl*)-*hydrazine*. 2,4-Dinitrofluorobenzene[47] (7 g, 37·5 mmoles) was refluxed for 1 hr with 7 g (37·5 mmoles) 1,1-diphenylhydrazine and 5·5 g (75 mmoles) sodium bicarbonate in 150 ml ethanol. Then 70 ml chloroform was added for solubilization of 2,4-ine and the solution was filtered and concentrated under reduced press. After cooling, filtering and washing with a small amount of ethanol, 12·5 g crude product m.p. 118° were obtained. Recrystallization from 450 ml ethanol afforded orange-red needles m.p. 120°, 10·5 g (Found: C, 61·8; H, 4·2; N, 16·1. $C_{18}H_{14}N_4O_4$ requires: C, 61·7; H, 4·0; N, 16·0%).

In the oxidation with lead peroxide in chloroform as described for 2,4,6-yl, a deep violet colour develops, and the solution affords an ESR signal, but attempts to isolate the radical in solid state through evaporation of the solvent under reduced press or dilution with ether failed: the solution became brown and deposited a brown material m.p. (dec.) 174° (from benzene–ligroin) which afforded no ESR signal. Its absorption spectrum showed bands at ca. 270, 340 and 440 mμ, with absorption intensities decreasing in this order. (Found: C, 61·05; H, 3·7; N, 14·75%).

Preparation of 1,1-*diphenyl*-2-(2,6-*dinitrophenyl*)-*hydrazine and* -*hydrazyl*. 2,6-Dinitrochlorobenzene[48] m.p. 78° (4·0 g, 20 mmoles) and 7·2 g (40 mmoles), diphenylhydrazine were refluxed for 40 hr in 150 ml ethanol. The brown-coloured product was filtered off, and purified through repeated recrystallizations from ethanol. On scratching, the orange-coloured 2,6-ine crystallized first, then a coffee-brown oxidation product, separated by rapid filtration. A better purification is achieved by chromatographic separation on alumina from benzene, 2,6-ine being eluted first, (5·5 g, 78%, m.p. 142–143°, deep orange (Found: C, 61·9; H, 4·0; N, 16·1. $C_{18}H_{14}N_4O_4$ requires: C, 61·7; H, 4·0; N, 16·0%), followed by the coffee-brown oxidation product. This crystallizes from ethanol in glistening leaflets m.p. 128° and yields orange-coloured solutions in 1,2-dichloroethane, whose absorption spectrum is nearly identical with the spectrum of 2,6-ine in neutral or basic medium (only the 400 mμ maximum is shifted with 10 mμ towards longer wavelengths). (Found: C, 60·8; H, 4·1; N, 14·9%). Its treatment with lead peroxide in dichloroethane affords a pink solution with absorption maxima at 254, 313 and 510 mμ.

Oxidation of 2,6-ine with lead peroxide in chloroform in the presence of sodium sulphate as described for 2,4,6-yl affords 2,6-yl in 95% yield. Recristallization from benzene–ligroin gives black-violet leaflets m.p. dec. 167°. Solutions in benzene, chloroform or dichloroethane are violet; only at very low concentrations ($2 \cdot 10^{-3}$ molar) can these solutions be discerned from 2,4,6-yl solutions of the same concentration (the latter are somewhat redder and were intensely coloured). (Found: C, 61·5; H, 3·7; N, 15·6; $C_{15}H_{13}N_4O_4$ requires: C, 61·9; H, 3·75; N, 16·0%).

Ultra-violet and visible absorption spectra were recorded at room temp with a Zeiss spectrophotometer.

Acknowledgements—We thank Professor C. D. Nenitzescu for his support and advice, and Miss Elvira Sliam, Miss Violeta Săndulescu and Miss Maria Petcu for microanalyses. Preliminary ESR measurements were performed by Mr. I. Pascaru.

[47] H. Zahn and A. Würtz, *Angew. Chem.* **63**, 147 (1951); *Z. Analyt. Chem.* **134**, 183 (1951).
[48] H. P. Schultz, *Org. Synth.* **31**, 45 (1951); F. D. Gunstone and S. H. Tucker, *Ibid.* **32**, 23 (1952).

Tetrahedron, 1961, Vol. 13, pp. 268 to 277. Pergamon Press, Inc. Printed in Northern Ireland

TOTALSYNTHETISCHE UNTERSUCHUNGEN AN STEROIDEN—VII[1]

EINFÜHRUNG DER C-SUBSTITUENTEN IN DIE ANGULARE STELLUNG DER KONDENSIERTEN RINGSYSTEME (TEIL 2). DEGRADATIVE DARSTELLUNG DER RAC. D-HOMO-18-NOR-ANDROSTAN-3β-OL-17-ON-13β- SOWIE 13α-CARBONSÄURE

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Zusammenfassung—C/D-*trans*- sowie *cis*-(13 → 17)-Laktamolmethyläther der rac. D-Homoandrostan-Reihe, III und IV, liessen sich in die entsprechenden N-Mesylderivate, V bzw. VI, überführen, die sich durch Einwirkung von verdünnter Natronlauge in wässrigem Dioxan unter Degradation in einer Ausbeute von ca. 90% in die 13β- bzw. 13α-Carbonsäure, XVIII bzw. XIX, verwandelten. Für den Mechanismus dieser Degradation ist eine Art von "1,4-Eliminierung unter Fragmentierung" denkbar, die neulich von C. A. Grob entwickelt wurde. Die hier eingeführten Reaktionsfolgen stellen eine Methode für die Einführung der angularen C-Substituent dar und liefern einen Anhaltspunkt zur Totalsynthese der normalen sowie der in 18-Stellung mit einer funktionellen Gruppe versehenen Steroide.

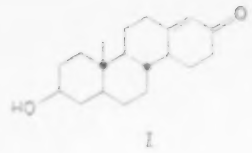
IN der vorangehenden Mitteilung[1] dieser Reihe ist eine Methode zur Darstellung von 13β- sowie 13α-Carbonsäureamiden (Teilformel II) der rac. D-Homoandrostanderivate beschrieben worden, die in der Einwirkung von Kaliumcyanid auf rac. D-Homo-18-nor-13(17a)-androsten-3β-ol-17-on (I) besteht. Es wurde ferner gezeigt, dass diese Säureamide im Gleichgewicht zum grössten Teil in der Hemiketalform IIb vorliegen und diese sich daher glatt in die entsprechenden Alkyläther (z.B. III und IV) überführen liessen.

Da diese an der angularen Stellung liegende Säureamid-Gruppe mit dem starr festgelegten C/D-kondensierten Ring ein Neopentyl-System[2] bildete, war eine grosse reaktionsträgheit der Carbonylgruppe gegen nukleophile Reagenzien vorauszu- sehen. In der Tat widerstanden diese der sauren sowie der alkalischen Verseifung unter gewöhnlichen Bedingungen vollständig und nur das Ausgangsmaterial wurde zurückerhalten. Unter drastischen Bedingungen fand starke Zersetzung statt und kein normales Verseifungsprodukt wurde isoliert. Zur Überwindung dieser Schwierigkeiten diente ein Modellversuch[3] am Cholestan-3-on-5-carbonsäureamid-hemiketal (VIII), wobei VIII über den Methyläther IX in das N-Tosyl oder N-Mesyl-derivat (X bzw. XI) übergeführt wurde und die letzteren sich durch Einwirkung von Natronlauge unter milden Bedingungen glatt in die entsprechende 5-Carbonsäure XII verwandelten.

[1] VI. Mitt. W. Nagata, T. Terasawa, T. Aoki und K. Takeda, Beitrag zur Zeitschrift *Chem. Pharm. Bull. Japan* im Druck.

[2] Zu Schwierigkeiten in der Verseifung des angularen Säurehydrazides, siehe, z.B., W. G. Dauben, R. C. Tweit und R. L. MacLean, *J. Amer. Chem. Soc.* 77, 48 (1955). vgl. noch M. S. Newman, *Steric Effects in Organic Chemistry* s. 73. John Wiley, New York (1956).

[3] W. Nagata, S. Hirai, H. Itazaki und K. Takeda, IV. Mitt. unter dem Titel *Über die angular substituierten polycyclischen Verbindungen* Beitrag zu *Liebigs Ann.* im Druck.



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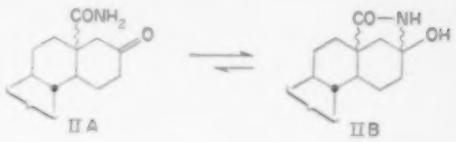
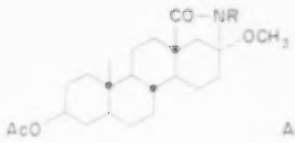
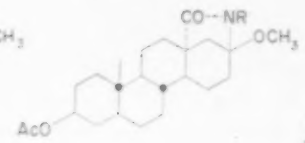


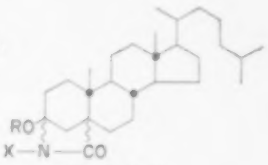
II A II B



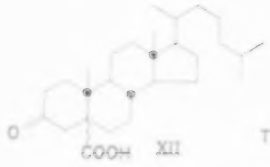
III R=H
V R=Ms



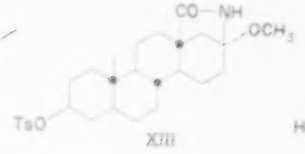
IV R=H
VI R=Ms
VII R=Ts



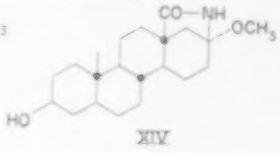
VIII R=H, X=H
IX R=CH3, X=H
X R=CH3, X=Ts
XI R=CH3, X=Ms



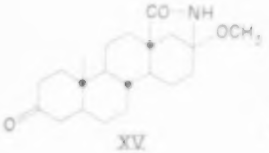
XII



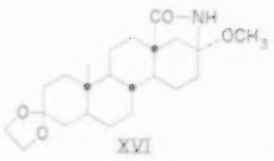
XIII



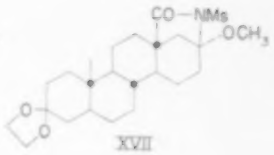
XIV



XV



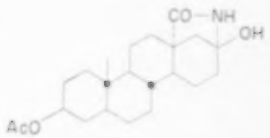
XVI



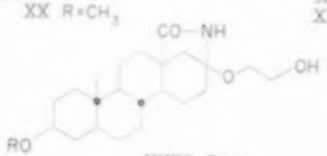
XVII

XVIII R=H
XX R=CH3

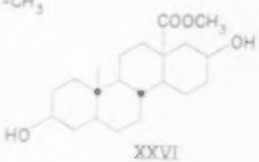
XIX R=H
XXI R=CH3



XXII

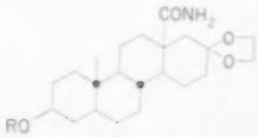


XXIII R=H
XXIV R=Ac



XXVI

XXVII

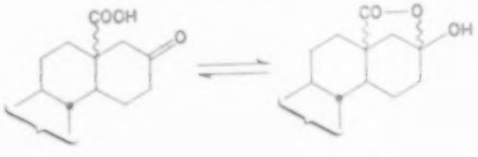


XXV

In Anlehnung an diesen Modellversuch liessen sich C/D-*trans*- und *cis*-Laktamolmethyläther, III und IV, mit Natriumhydrid oder Lithiumamid und *p*-Toluolsulfonsäurechlorid tosylieren, wobei aber die Umsetzungen im Gegensatz zum Modellversuch sehr schlecht verliefen. Zwar ergab die C/D-*cis*-Verbindung IV das erwünschte N-Tosylat VII, aber nur in schlechter Ausbeute und das Ausgangsmaterial wurde zum grössten Teil regeneriert. Andererseits fand bei der Umsetzung vom C/D-*trans*-Körper III auffallenderweise keine N-Tosylierung, sondern eine O-Tosylierung an der 3β-Hydroxylgruppe statt, wenn auch nur in geringer Ausbeute. Zum Beweis dafür diente das IR-Spektrum des Produktes XIII, das noch das Vorliegen der unveränderten Imid-Gruppe aber keine Acetoxylgruppe mehr aufwies.* Ferner war die einzige Carbonylbande bei 1702 cm^{-1} gar nicht nach kürzeren Wellen verschoben, was gegen ein N-Tosylsäureamid spricht.[3] Da XIII ausserdem durch Reduktion mit Lithium und Alkohol in wässrigem Ammoniak glatt in den freien Alkohol XIV überführt wurde, ist die Formulierung XIII für dieses Umsetzungsprodukt einwandfrei richtig.

Im Gegensatz zur Tosylierung verlief die Mesylierung sehr glatt. Die Umsetzung von III und IV mit Natriumhydrid und Methansulfonsäurechlorid in der Toluol- oder Xylol-Lösung ergab nun die erwünschten Produkte V und VI in einer Ausbeute von 83–87% bzw. 56–58%, bezogen auf das gebrauchte Ausgangsmaterial. Die Ergebnisse der Mesylierung der Verbindung IV sind in der Tabelle 1 zusammengestellt. Ferner wurde C/D-*trans*-3-Keto-laktamolmethyläther XV wie üblich ketalisiert und das Ketal XVI wurde ebenfalls mit NaH und Mesylchlorid glatt mesyliert (XVII).

Nun wurden C/D-*trans*- und *cis*-N-Mesyl-laktamolmethyläther V und VI in der Dioxan-Lösung einer alkalischen Hydrolyse unterworfen. Parallel dem Modellversuch verlief die Reaktion sehr glatt und V sowie VI liessen sich durch Erhitzen mit verdünnter Natronlauge in Dioxan in guter Ausbeute in die entsprechenden 13β- sowie 13α-Carbonsäuren, XVIII und XIX verwandeln, die durch Methylierung mit Diazomethan glatt in die Methylester XX bzw. XXI übergeführt wurden. Die Ausbeute an den Estern XX und XXI konnte man, bezogen auf den N-Mesylkörper, mit 87·5% der Theorie für den ersteren und 93·4% für den letzteren berechnen, wenn man, ohne die intermediär gebildete freie Säure rein zu isolieren, die Operation durchführt. Es war ferner bemerkenswert, dass das IR-Spektrum von 13β-Carbonsäure XVIII in festem Zustand eine Bande für γ-Lakton bei 1733 cm^{-1} und keine Bande für das sechsgliedrige Ringketon mehr aufweist, während dasjenige von 13α-Carbonsäure XIX normalerweise Banden für Ringketon und Carbonsäure bei 1711 cm^{-1} bzw. bei 1691 cm^{-1} zeigt. Dies deutet darauf hin, dass die Säure XVIII im Gegensatz zu XIX in festem Zustand weitgehend in der Brückenform B vorliegt. Der Grund dafür dürfte ebenfalls[1] in der durch die *cis*-Verknüpfung bedingten größeren Biegsamkeit der

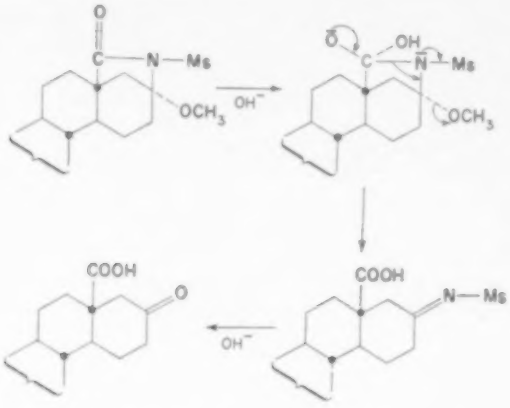


A B

Konformation des C/D-Ringes bei XIX zu suchen sein.

* s. experimenteller Teil.

Betreffs des Mechanismus dieser Degradationsreaktion könnte man annehmen, dass es sich um eine Art der äthylogen 1,4-Eliminierung unter Fragmentierung,[3,4] unter einem synchronen Prozess handelt, weil die hier eingeordneten Atomgruppen geometrisch sowie räumlich dazu sehr geeignet liegen. Demnach verläuft die Reaktion



folgenderweise (Schema 3), wobei sich die Reaktivität der Carbonylgruppe gegen das nukleophile Hydroxylion, durch die Elektronen anziehende Wirkung des Mesyl-Radikals, in hohem Masse verstärken lässt.

Auf diese Weise konnte der Substituent mit der Carbonylfunktion in die angulare C_{13}-Stellung sehr glatt eingeführt werden, was zur Synthese der 18-oxygenierten Steroide, wie Aldosteron, von grosser Wichtigkeit ist.

Ferner wurde C/D-*cis*-Laktamol XXII[1] wie üblich ketalisiert und nach einer Al_2O_3-chromatographischen Trennung wurden zwei Produkte erhalten. Gemäss den I.R.-Spektren, sowie den Analysenwerten erwies sich das zuerst eluierte Produkt als ein 17β-Hydroxy-äthylätheracetat XXIV und das andere als dessen teilweise verseifter 3β-Alkohol XXIII. Für den Ausschluss der Formel XXV, die ein normales Ketal aufweist, gelten auch hier die in der vorhergehenden Mitteilung[5] angegebenen Beweise. XXIV wurde dann mit einer dreifachen Menge von NaOH in Äthylenglykol 4 Stunden auf 120–200° erhitzt, wobei eine langsame Ammoniak-Entwicklung beobachtet wurde. Nach der anschliessenden Methylierung wurde das Rohprodukt einer Al_2O_3-Chromatographie unterworfen, wonach neben einer geringen Menge eines unbekannten Produktes (WN 251), ein Produkt vom Schmp. 222–227° in einer Ausbeute von nur ca. 17% erhalten wurde. Nach dem IR-Spektrum und den Analysenwerten handelt es sich bei ihm um einen Dihydroxy-carbonsäureester XXVI, dessen 17-Hydroxylgruppe aus dem ursprünglichen 17-Keton durch Einwirkung des Natriumglykolates nach einer Art von Meerwein-Ponndorf'scher Reduktion entstanden ist. Da unter dieser drastischen Einwirkung von Base die Hydroxylgruppe eine äquatoriale, hierbei also β-Konfiguration annehmen sollte, konnte man nun dieser die Formel XXVI zukommen lassen.[6] Dies wurde weiter dadurch bestätigt, dass sich XXVI durch

[4] C. A. Grob und W. Baumann, *Helv. Chim. Acta* **38**, 594 (1955), C. A. Grob, *Experientia* **13**, 126 (1957).
[5] W. Nagata, S. Hirai, H. Itazaki und K. Takeda, I. Mitteilung unter dem Titel *Über die angular substituierten polycyclischen Verbindungen* Beitrag zu *J. Org. Chem.* im Druck
[6] vgl. W. Nagata, S. Hirai, T. Aoki und K. Takeda, III. Mitteilung unter dem Titel *Über die angular substituierten polycyclischen Verbindungen* Beitrag zu *Chem. Pharm. Bull.* im Druck.

Oxydation mit CrO_3-Pyridin-Komplex in einen Diketon-Ester überführen liess, der sich mit dem aus dem oben erwähnten *cis*-Ketol-Ester XXI durch gleiche Behandlung erhaltenen *cis*-Diketon-Ester XXVII in jeder Hinsicht als identisch erwies.

BESCHREIBUNG DER VERSUCHE

Alle Schmp. sind auf dem Kofler-Block "Monoskop" (Fa. Hans Bock, Frankfurt/Main, Deutschland) bestimmt und korrigiert worden. Alle Sdp. sind unkorrigiert. Wenn nicht anders angegeben, wurden Substanzproben zur Elementaranalyse 3 Std. bei 1 Torr über P_2O_5 bei Zimmertemperatur bis zu 60°, 70–90° und 100–120° je nach dem Schmp. bis zu 120°, 180° und über 180° getrocknet. Die adsorptionschromatogramme wurden nach der Durchlaufmethode[7] durchgeführt. Es werden folgende Abkürzungen benützt; Pe = Petroläther, Ae = Äther, Alk = Alkohol, An = Aceton, Bzl = Benzol, Chf = Chloroform, Me = Methanol, Pn = Pentan, Py = Pyridin, Akt. = Aktivität.

I C/D-Trans-*Reihe*

A (1) *Ketalisierung von* XV[1]

300 mg XV wurden üblicherweise mit 80 mg Äthylenglykol und 50 cc abs. Bzl in Gegenwart von 30 mg *p*-Toluolsulfonsäuremonohydrat ketalisiert. Die übliche Aufarbeitung ergab 409 mg Eindampfrückstand, der direkt aus Chf-An umkristallisiert wurde. Dieser ergab 268 mg rohes XVI vom Schmp. 265–273°. Die Mutterlaugen wurden nicht weiter aufgearbeitet. Zur Analyse wurde rohes XVI weiter aus Chf-An umkristallisiert; reines XVI schmolz bei 267–273°.

dl-D-*Homo*-18-*nor*-17α-*methoxy*-17β-*aminoandrostan*-3-*on*-13β-*carbonsäure*-(13 → 17)β-*laktam*-3-*äthylenketal* XVI. Pyramiden, Schmp. 267–273°, I.R. ν_{max}^{Chf} cm^{-1} 3455 (NH), 1705 (—CO—NH—), 1111, 1086 (C—O), (Gef.: C, 70·72; H, 9·00; N, 3·61. $C_{23}H_{35}O_4N$ (M.G. 389·52) Ber.: C, 70·92; H, 9·06; N, 3·60%).

A (2) N-*Mesylierung von* XVI

50 mg XVI wurden in einem Gemisch von 7·5 cc abs. Xylol und 2·5 cc abs. Dioxan gelöst, durch azeotrope Destillation (ca. 3 cc Destillat) wurde das anwesende wasser entfernt. In diese Lösung wurden 6 mg NaH eingetragen und unter einem N_2-Strom und Rühren 5 Std. lang unter Rückfluss gekocht, wozu eine Lösung von 44 mg MsCl in 4 cc abs. Xylol zugetropft und dann weiter 2·5 Std. bis zum Sieden erhitzt wurde.

Nach der Abkühlung wurde die organische Phase abgetrennt und die wässrige Phase zweimal mit Chf extrahiert. Die organischen Schichten ergaben nach Waschen mit Natriumsulfat-Lösung, Trocknen über Na_2SO_4 und Eindampfen im Vakuum 85·9 mg Rohprodukt, das an 2·5 g Al_2O_3 chromatographiert wurde. Die mit Pe-Bzl (3:1)-Bzl-Chf (9:1) eluierten Fraktionen (Nr. 6–11) ergaben aus Chf-An 27·0 mg XVII in Nadeln vom Schmp. 275–284°, dessen Schmp. durch nochmalige Umkristallisation bis auf 282–284·5° erhöht wurde. Die nachfolgenden Fraktionen (Bzl–Chf 9:1–Chf, Nr. 12–20) ergaben aus Chf–An 8·6 mg Ausgangsmaterial (XVI) vom Schmp. 268–276° (Mischprobe).

dl-D-*Homo*-18-*nor*-17α-*methoxy*-17β-*mesylaminoandrostan*-3-*on*-13β-*carbonsäure*-(13 → 17)β-*laktam*-3-*äthylenketal* XVII. Nadeln aus Chf-An, Schmp. 282–284·5°, I.R. ν_{max}^{Chf} cm^{-1} 1741 (—CO—N—Ms), 1363, 1150 (SO_2—N—), (Gef.: C, 61·19; H, 8·04; N, 3·14; S, 6·74. $C_{24}H_{37}O_6SN$ (M.G. 467·54) Ber.: C, 61·65; H, 7·98; N, 3·00; S, 6·85%).

A (3) *Tosylierung von* III.

(i) Zu einer Suspension von $LiNH_2$ in 10 cc abs. Ae, hergestellt aus 18 mg Li und 20 cc flüssigem NH_3, das im voraus über Li getrocknet und dann nochmals destilliert wurde, wurden 20 cc einer Mischung von abs. Toluol und abs. Xylol (1:1 v/v) zugegeben und der Ae wurde abdestilliert. Dazu wurde unter einem N_2-Strom sowie Rühren eine Lösung von 500 mg III in 16·5 cc desselben Lösungsmittels zugetropft und 5 Std. unter Rückfluss gekocht. Hierauf wurde eine Lösung von 490 mg *p*-Toluolsulfonsäurechlorid in 10 cc abs. Xylol zugetropft und dann 1·5 Std. bis zum Sieden erhitzt. Nach Abkühlen wurde etwas Wasser zugegeben. Die organische Phase wurde abgetrennt und die wässrige zweimal mit Chf extrahiert. Die organischen Schichten ergaben nach dem Waschen mit

[7] T. Reichstein und C. W. Shoppee, *Disc. Faraday Soc.* Nr. 7, 305 (1949).

Na_2SO_4-Lösung, Trocknen über Na_2SO_4 und Eindampfen 850 mg Rückstand, der an 30 g Al_2O_3 chromatographiert wurde. Die mit Bzl–Chf (9:1–8:2) eluierten Fraktionen (Nr. 13–20) ergaben aus Me-An 205·4 mg Ausgangsmaterial III (Mischprobe) und dann folgten aus den mit Bzl–Chf (8:2–1:1) eluierten Fraktionen (Nr. 21–25) aus An–Ae 117 mg XIII in Prismen vom Schmp. 197–198°. Zuletzt wurden aus den mit Bzl–Chf (1:2)—Chf eluierten Fraktionen (Nr. 30–33) 11·5 mg XIV vom Schmp. 237–238° (Mischprobe) erhalten, welche mit Ac_2O und Py zum Ausgangsmaterial III acetyliert wurde (s. unten).

(ii) 205 mg III wurden gleicherweise wie bei A–2 mit 18 mg NaH und 150 mg TsCl umgesetzt, nur dass eine Mischung von abs. Xylol und wasserfreiem Dioxan (1:1) als Lösungsmittel verwendet wurde. Der nach analoger Aufarbeitung erhaltene Eindampfrückstand (293·8 mg) wurde ebenfalls an 8 g Al_2O_3 chromatographiert, wonach erhalten wurden: aus den Fraktionen Nr. 14–25 (Bzl–Chf, 9:1–8:2) 51·2 mg Ausgangsmaterial (III), aus den Fraktionen Nr. 26–30 (Bzl–Chf, 8:2–1:1) 8·9 mg XIII vom Schmp. 195–197° und aus den Fraktionen Nr. 37–45 (Chf und Chf–Me, 99·5:0·5) etwas vom verseiften Ausgangsmaterial (XIV) vom Schmp. 233–235°, das mit demselben aus (i) vereinigt (78·7 mg) und mit 3 cc Py und 2 cc Ac_2O bei Raumtemperatur über Nacht acetyliert wurde. Das erhaltene Acetat schmolz allein sowie zusammen mit III bei 263–265°.

dl-D-*Homo*-18-*nor*-17α-*methoxy*-17β-*aminoandrostan*-3β-ol-13β-*carbonsäure*-(13 → 17)β-*laktam*-3-*tosylat* XIII. Prismen aus An und Ae, Schmp. 197–198°, I.R. ν_{max}^{Chf} cm^{-1} 3475 (NH), 1702 (—NH—CO—), 1603, 1495 (Phenyl), 1370, 1358, 1179 (SO_2N—), (Gef.: C, 67·38; H, 8·09; N, 2·99; S, 5·88. $C_{28}H_{39}O_5NS$ (M.G. 501·60) Ber.: C, 67·04; H, 7·84; N, 2·79; S, 6·38%).

A (4) *Reduktion von* XIII *mit Li in flüssigem Ammoniak*

Zu einer Lösung von 250 mg Li in 15 cc flüssigem NH_3 wurde unter Rühren eine Lösung von 50 mg XIII in 4 cc abs. Dioxan und 6 cc abs. Ae innerhalb von 10 Min. hinzugetropft. Nach 5 Min. wurde mit 3 cc abs. Alk versetzt und dann das Ammoniak verdunstet. Der Rückstand wurde mit etwas Wasser versetzt, mit wenig 2N HCl auf pH 7–8 gebracht und dann dreimal mit Chf extrahiert. Die Chf-Auszüge ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vakuum 53 mg Eindampfrückstand, der an 2 g Al_2O_3 chromatographiert wurde. Die mit Bzl–Chf (1:1) eluierten Fraktionen (Nr. 8–14) ergaben aus An–Ae 13·5 mg XIV in Prismen vom Schmp. 239–240°, aus der Mutterlauge weitere 5 mg vom Schmp. 236–241°, die sich nach einer Mischprobe sowie dem Vergleich der IR-Spektren als identisch mit dem authentischen Präparat von XIV[1] erwies

A (5) N-*Mesylierung von* III

(i) In Toluol. 380 mg III wurden in 60 cc abs. Toluol gelöst, durch azeotrope Destillation (ca. 10 cc Destillat) wurde das anwesende Wasser entfernt. Dazu wurden 48 mg (2 Mol Äquiv.) NaH zugegeben und dann unter einem N_2-Strom sowie Rühren eine halbe Std. unter Rückfluss gekocht. Hierauf wurde eine Lösung von 230 mg (2 Mol Äquiv.) MsCl in 10 cc abs. Toluol innerhalb einer halben Std. zugetropft und weitere 2 Std. bis zum Sieden erhitzt. Unter Abkühlung mit Eiswasser wurden 15 cc Wasser vorsichtig zugetropft und die organische Phase abgetrennt. Die wässrige Phase wurde dreimal mit Chf extrahiert und die organischen Auszüge wurden mit Wasser gewaschen, über Na_2SO_4 getrocknet und dann im Vakuum eingedampft. Der so erhaltene Eindampfrückstand (470 mg) wurde mit 3 cc Ac_2O und 5 cc Py bei Raumtemperatur über Nacht zurückacetyliert. Die übliche Aufarbeitung ergab 473 mg eines kristallinischen Rohproduktes, das aus Chf–An–Ae umkristallisiert wurde. Es wurden 246 mg V in dünnen langen Platten vom Schmp. 249–254° erhalten. Die erste Mutterlauge wurde im Vakuum eingedampft und der Rückstand (235 mg) wurde an 8 g Al_2O_3 chromatographiert. Die mit Pe–Bzl (4:6)–Bzl eluierten Fraktionen (Nr. 10–25) ergaben aus Chf–An weitere 61·4 mg V vom Schmp. 256–259° und die mit Bzl–Chf 9:1–1:1 eluierten Fraktionen (Nr. 27–34) ergaben aus Chf–An 76·7 mg des Ausgangsmaterials III (Mischprobe) in Nadeln. Die Ausbeute von V betrug insgesamt 307·4 mg (83·5% bezogen auf das gebrauchte Ausgangsmaterial).

(ii) in Toluol–Xylol (1:1). 312·4 mg III wurden analog wie bei A–5 (i) mit 38 mg NaH und 185 mg MsCl umgesetzt, wobei anstatt abs. Toluol eine Mischung von Toluol und Xylol im Volumenverhältnis 1:1 als Lösungsmittel verwendet wurde. Die analoge Aufarbeitung und anschliessende Zurückacetylierung ergaben aus An–Ae 201·8 mg V vom Schmp. 251–258°. Die Mutterlauge (202 mg) ergab nach der Chromatographie an 8 g Al_2O_3 weitere 67·5 mg vom Schmp. 260–263° (aus den

Fraktionen Nr. 8–24, Pe–Bzl (1:2)–Bzl) und 52·4 mg des Ausgangsmaterials III vom Schmp. 259–262° (aus den Fraktionen Nr. 26–28, Bzl–Chf (75:25)). Die Ausbeute von V war dann 269·3 mg (87% bezogen auf das gebrauchte III).

dl-D-*Homo*-18-*nor*-3β-*acetoxy*-17β-*mesylamino*-17a-*methoxyandrostan*-13β-*carbonsäure*-(13 → 17)-β-*laktam* V. Dünne lange Platten aus An–Ae, Schmp. 260–263°, I.R. ν_{max}^{Chf} cm^{-1} 1730 (OC—N—, CH_3COO—), 1356, 1169, 1155, 1141 (SO_2—N—), 1248, 1028 (CH_3COO—), (Gef.: C, 61·45; H, 8·03; N, 2·91; S, 7·05. $C_{24}H_{37}O_6NS$ (M.G. 467·54) Ber.: C, 61·65; H, 7·98; N, 3·00; S, 6·89%).

B (1) *Degradation von* V *mit Natronlauge*

Eine Mischung von 30 mg V und 100 mg NaOH in 3·3 cc wasserfreiem Dioxan und 0·65 cc Wasser wurden unter einem N_2-Strom 1 Std. bis zum Sieden erhitzt. Nach der Abkühlung wurde die Reaktionslösung mit 150 mg Essigsäure neutralisiert und im Vakuum eingeengt. Unter Abkühlung wurde der Rückstand mit etwas Wasser versetzt und mit konz. HCl angesäuert. Die ausgeschiedenen Kristalle wurden dreimal mit Chf extrahiert, zweimal mit Wasser gewaschen, über Na_2SO_4 getrocknet und dann im Vakuum eingedampft. Der Rückstand (23·2 mg) ergab aus An und Ae 16·8 mg Säure XVIII vom Schmp. gegen 310° unter Zersetzung, die nochmals aus Me (ein wenig), Chf und An umkristallisiert wurde, jedoch keine Schmp.-Erhöhung zeigte.

dl-D-*Homo*-18-*nor*-*androstan*-3β-*ol*-17-*on*-13β-*carbonsäure* XVIII. Mikroblätter aus Chf–An, Schmp. gegen 310° unter Zersetzung. I.R. ν_{max}^{Nujol} cm^{-1} 3540, 3265(OH), 1733(CO—O), 1710(Inflexion, COOH, C—O), (Gef.: C, 71·82; H, 9·14. $C_{20}H_{30}O_4$ (M.G. 334·44) Ber.: C, 71·82; H, 9·04%).

B (2) *Behandlung von* XVIII *mit Diazomethan*

85·7 mg XVIII wurden in 6 cc Dioxan gelöst und dann mit Diazomethan in ätherischer Lösung methyliert. Die übliche Aufarbeitung ergab 91·8 mg Rohprodukt, das aus An–Ae 82·1 mg Ester XX in Säulen vom Schmp. 186–187° ergab. Nach weiterer Umkristallisation schmolz der reine Ester bei 193–194°.

dl-D-*Homo*-18-*nor*-*androstan*-3β-*ol*-17-*on*-13β-*carbonsäuremethylester* XX. Säulen aus An und Ae, Schmp. 193–194°, I.R. ν_{max}^{Nujol} cm^{-1} 3487 (OH), 1731 (Ester), 1703 (C—O), 1415 (CH_2CO—), (Gef.: C, 72·78; H, 9·32. $C_{21}H_{32}O_4$ (M.G. 348·47) Ber.: C, 72·38; H, 9·26%).

B (3) *Degradation und anschliessende Methylierung*

Eine Mischung von 1·45 g V und 4·5 g NaOH in 150 cc wasserfreiem Dioxan und 29 cc Wasser wurde unter einem N_2-Strom 3 Std. bis zum Sieden erhitzt. Nach der Abkühlung wurde die Reaktionslösung mit 5·4 g Eisessig angesäuert und dreimal mit Chf extrahiert. Die Chf-Extrakte wurden dreimal mit 2N NaOH ausgeschüttelt, zweimal mit Wasser gewaschen, über Na_2SO_4 getrocknet und dann im Vakuum eingedampft. Der erhaltene neutrale Eindampfrückstand (278 mg) ergab aus An-Ae total 253 mg rohes Ausgangsmaterial (V) vom Schmp. 242–250° sowie aus der Mutterlauge noch etwas V vom Schmp. 224–234° (Mischprobe).

Die alkalische Lösung wurde mit dem Waschwasser vereinigt und unter Abkühlung mit Eiswasser mit konz. Salzsäure bis auf Kongograu angesäuert. Die ausgeschiedenen Kristalle wurden sechsmal mit dem Misch-Lösungsmittel von Chf–Me (9:1 v/v) erschöpfend ausgeschüttelt. Die organische Phase ergab nach dem Waschen mit Wasser, dem Trocknen über Na_2SO_4 und dem Eindampfen im Vakuum 863 mg des sauren Anteiles, der ohne weiteres in der Dioxan-Lösung mit Diazomethan methyliert wurde. Der erhaltene rohe Ester (896·2 mg) ergab aus An–Ae 575·5 mg XX vom Schmp. 192–195°, 151·3 mg vom Schmp. 186–189° und 51·0 mg vom Schmp. 178–182°. Insgesamt 777·8 mg (87·5% bezogen auf das Ausgangsmaterial).

II C/D-Cis-*Reihe*

A (1) *Tosylierung von* IV

79·3 mg IV wurden in 10 cc abs. Toluol gelöst, durch Destillation (ca. 5 cc Destillat) wurde das anwesende Wasser entfernt. Zu dieser Lösung wurden unter einem N_2-Strom sowie Rühren 16 mg NaH zugegeben. Dann wurde 2 Std. unter Rückfluss gekocht und darauf eine Lösung von 127 mg *p*-Toluolsulfonsäurechlorid in 5 cc abs. Toluol zugetropft; anschliessend weitere 2 Std. bis zum Sieden erhitzt. Unter Abkühlung mit Eiswasser wurde die Lösung vorsichtig mit Wasser versetzt und die organische Phase abgetrennt. Die wässrige Phase wurde dreimal erschöpfend ausgeäthert. Die organischen Auszüge wurden mit Wasser gewaschen, über Na_2SO_4 getrocknet und dann im Vakuum

eingedampft. Das erhaltene Rohprodukt (215·8 mg) wurde an 8 g Al_2O_3 chromatographiert. Die mit Bzl–Chf (8:2) eluierten Fraktionen (Nr. 25–29) ergaben aus Ae und Pn 16·1 mg Tosylat VII vom Schmp. 178–184° und die mit Bzl–Chf (1:1–1:2) eluierten Fraktionen aus An–Ae 17·4 mg Ausgangsmaterial (Mischprobe).

dl-D-*Homo*-18-*nor*-3*β*-*acetoxy*-17*β*-*methoxy*-17*α*-*tosylaminoandrostan*-13*α*-*carbonsäure*-(13 → 17)-*α*-*laktam* VII. Schmp. 178–184°, I.R. ν_{max}^{Chf} cm^{-1} 1729 (AcO, —CO—N—), 1600, 1492 (phenyl), 1364, 1177 (SO_2—N—), 1249, 1026 (AcO), (Gef.: C, 66·12; H, 7·64; N, 2·69. $C_{30}H_{41}O_6NS$ (M.G. 543·64) Ber.: C, 66·28; H, 7·60; N, 2·58%).

A (2) N-*Mesylierung von* IV

275 mg IV wurden in 55 cc abs. Xylol gelöst und das anwesende Wasser als azeotropes Destillat (5 cc) entfernt. 40 mg NaH wurden zugegeben und dann unter einem N_2-Strom und Rühren 1 Std. zum Sieden erhitzt, worauf eine Lösung von 190 mg Mesylchlorid in 10 cc wasserfreiem Xylol zugetropft und 3 Std. weiter unter Rückfluss gekocht wurde.

Unter Abkühlung wurde die Reaktionslösung vorsichtig mit Wasser verserzt und dann mit Chf extrahiert. Die Extrakte wurden nach Waschen mit Wasser über Na_2SO_4 getrocknet und dann im Vakuum eingedampft. Der erhaltene Rückstand (329 mg) wurde mit 3 cc Ac_2O und 5 cc Py bei Raumtemperatur über Nacht acetyliert. Das rohe Acetat (319 mg) wurde an 9 g Al_2O_3 chromatographiert. Die mit Pe–Bzl (6:4)–Bzl–Chf (7:3) eluierten Fraktionen (Nr, 5–22) ergaben aus An–Ae insgesamt 159 mg (48·1%, oder 56·4% bezogen auf das gebrauchte IV) rohes VI in Würfeln vom Schmp. 206–212° bzw. Schmp. 202–212°; die mit Bzl–Chf (4:6)–Chf eluierten Fraktionen (Nr. 24–29) ergaben aus An-Ae insgesamt 40·2 mg Ausgangsmaterial IV.

Die unter verschiedenen Reaktionsbedingungen gewonnenen Resultate wurden in Tabelle 1. zusammengestellt.

TABELLE 1. MESYLIERUNG VON IV

Nr.	IV (mg.)	NaH (Äq. Mol)	MsCl (Äq. Mol)	Lösungsm. (cc) Tol[a]	Xyl[b]	Tol-Xyl[c]	Reaktionsdauer (Rückfluss)	Ausbeute von VI % (%[d])	Ausgangsmaterial IV %
1	275	2·36	2·36	60			3	48·1 (56·4)	14·6
2	390	2·10	2·00	60			2·5	37·8 (58·8)	35·8
3	600	2·00	2·00	60			2·5	33·2 (44·6)	25·5
4	390	2·00	2.00			60	2·5	24·8 (58·5)	57·7
5	160	2·00	2·00	25			3·5	32 (39·0)	17·5

[a] Tol = Toluol, [b] Xyl = Xylol, [c] Tol-Xyl = Toluol-Xylol (1:1 v/v)
[d] bezogen auf das Ausgangsmaterial (IV).

dl-D-*Homo*-18-*nor*-3*β*-*acetoxy*-17*β*-*methoxy*-17*α*-*mesylaminoandrostan*-13*α*-*carbonsäure*-(13 → 17)-*α*-*laktam* VI. Würfel aus An und Ae, Schmp. 206–212°, I.R. ν_{max}^{Chf} cm^{-1} 1730 (—CO—N—, AcO), 1360, 1173 (SO_2—N—), 1261, 1028 (AcO), (Gef.: C, 61·82; H, 8·13; N, 3·20; S, 6·61. $C_{24}H_{37}O_6NS$ (M.G. 467·54) Ber.: C, 61·65; H, 7·98; N, 3·00; S, 6·85%).

B (1) *Degradation von* IV *mit Natronlauge*

120 mg IV wurden in 10 cc Dioxan gelöst, mit einer Lösung von 400 mg NaOH in 2·5 cc Wasser versetzt und dann unter einem N_2-Strom eine Std. bis zum Sieden erhitzt. Nach der Abkühlung wurde mit 0·6 cc Eisessig neutralisiert und im Vakuum eingeengt. In der Kälte wurde der Rückstand mit konz. HCl bis auf Kongograu angesäuert und dann dreimal mit Chf extrahiert. Die Chf-Auszüge ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4 und nach Eindampfen im Vakuum einen kristallinischen Rückstand, der aus Chf–An–Ae 76·7 mg freie Säure (XIX) in Säulen vom Schmp. 250–254°, (ca. 90%) ergab. Weiteres Umkristallisieren erhöhte ihren Schmp. bis auf 251–255°. (Sie zersetzte sich bei 300° unter Gas-Entwicklung). Die weitere Reinigung (Umlösen in 2N NaOH, mehrfaches Waschen mit Chf, Ansäuren mit konz. HCl, dann Extrahieren mit Chf) ergab keine Schmp. Erhöhung.

dl-D-*Homo*-18-*nor*-*androstan*-3*β*-*ol*-17-*on*-13*α*-*carbonsäure* XIX. Säulen aus Chf-An-Ae, Schmp. 251–255°/300° (Zers.), I.R. ν_{max}^{Nujol} cm^{-1} 3525 (OH), 2500–2700 (breite Bande COOH), 1711 (C=O), 1691 (COOH), (Gef.: C, 71·46; H, 9·07. $C_{20}H_{30}O_4$ (M.G. 334·44) Ber.: C, 71·82; H, 9·04%).

B (2) *Methylierung von* XIX *mit Diazomethan*

43·7 mg Carbonsäure XIX wurden wie üblich mit Diazomethan methyliert und aufgearbeitet. Das Rohprodukt (44·1 mg) ergab aus An–Ae 36·7 mg (81·5%) XXI in dicken Prismen vom Schmp. 154–158°, die nach weiterer Umkristallisation bei 164–167° schmolzen.

dl-D-*Homo*-18-*nor*-*androstan*-3*β*-*ol*-17-*on*-13*α*-*carbonsäuremethylester* XXI. Prismen aus An-Ae, Schmp. 164–167°, I.R. ν_{max}^{Chf} cm^{-1} 3629 (OH), 1728 (Ester), 1710 (C=O), 1142 (Ester), (Gef.: C, 72·06; H, 9·29. $C_{23}H_{36}O_4$ (M.G. 348·47) Ber.: C, 72·38; H, 9·26%).

B (3) *Degradation von* XIX *und anschliessende Methylierung*

587 mg IV wurden in 45 cc Dioxan gelöst, mit einer Lösung von 1·9 g NaOH in 12 cc Wasser versetzt und dann unter einem N_2-Strom 1·5 Std. bis zum Sieden erhitzt. Nach der Abkühlung wurde die Reaktionslösung bis auf pH 8 neutralisiert, im Vakuum eingeengt und dreimal mit Chf ausgeschüttelt. Die Chf-Auszüge wurden erst mit 2N NaOH, dann zweimal mit Wasser ausgeschüttelt und über Na_2SO_4 getrocknet. Abdampfen des Chf im Vakuum ergab 15·0 mg neutralen Rückstand (verworfen). Die wässrigen Schichten wurden vereinigt, in der Kälte mit konz. HCl bis auf Kongograu angesäuert und fünfmal mit Chf extrahiert. Die Chf-Auszüge ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vakuum 463 mg kristallinischen Rückstand, der ohne weiteres in Dioxanlösung mit CH_2N_2 methyliert wurde. Die übliche Aufarbeitung ergab 480 mg rohen Ester, der aus An–Ae 314·4 mg reinen Ester XXI in Prismen vom Schmp. 158–161°, aus der Mutterlauge weitere 93·6 mg vom Schmp. 158–160° (insgesamt 408 mg; 93·4% aus XII) ergab.

B (4) *Oxydation von* XXI

100 mg XXI wurden in eine Suspension von CrO_3-Py-Komplex in Py, hergestellt aus 200 mg CrO_3 und 2 cc Py, in der Kälte eingetragen und dann bei Raumtemperatur 5 Std. stehen gelassen. Die übliche Aufarbeitung ergab 94·8 mg kristallinisches Produkt, das aus An–Ae 69·2 mg Diketon (XXVI) vom Schmp. 178·5–180·5° in Platten und aus der Mutterlauge weitere 15·9 mg vom Schmp. 175–178° (total 85·1 mg, 85·6%) ergab. Weitere Umkristallisation der ersten Kristalle erhöhte den Schmp. auf 180–181°.

dl-D-*Homo*-18-*nor*-*androstan*-3,17-*dion*-13*α*-*carbonsäuremethylester* XXVI. Platten aus An–Ae, Schmp. 180–181°, I.R. $\nu_{max}^{CH_2Cl_2}$ cm^{-1} 3460 (schwach, Oberton von CO?), 1730 (Ester), 1710 (C=O), (Gef.: C, 73·05; H, 8·76. $C_{23}H_{34}O_4$ (M.G. 346·45) Ber.: C, 72·80; H, 8·73%).

C (1) *Herstellung von* 17-(*β*-*Hydroxyäthyl*)-*äther* (XXIII *und* XXIV)

328·5 mg XXII wurden mit 0·7 cc Äthylenglykol in 120 cc abs. Bzl in Gegenwart von 40 mg *p*-Toluolsulfonsäuremonohydrat üblicherweise unter Verwendung des Wasser-Abscheiders ketalisiert (Reaktionsdauer ca. 16 Std.). Die übliche Aufarbeitung ergab 428·3 mg rohes Produkt, das an 12 g Florisil chromatographiert wurde. Die mit Chf und Chf–Me (99:1) eluierten Fraktionen (Nr. 24–31) ergaben aus An–Ae 170 mg XXIV in dünnen Platten vom Schmp. 183–187°, nach weiterem Umkristallisieren Schmp. 191–193°. Die mit Chf–Me (98:2)-(90:10) eluierten Fraktionen ergaben aus An–Ae 82·8 mg rohes XXIII in Prismen vom Schmp. 240–246°, nach weiterem Umkristallisieren, vom Schmp. 250–257°.

dl-D-*Homo*-18-*nor*-3*β*-*acetoxy*-17*α*-*amino*-17*β*-(*β*-*hydroxyäthoxy*)-*androstan*-13*α*-*carbonsäure*-(13 → 17)*α*-*laktam* XXIV. Dünne Platten aus An–Ae, Schmp. 191–193°, I.R. ν_{max}^{Nujol} cm^{-1} 3556 (OH), 3320, 3236 (NH), 1727 (AcO), 1688 (CO–NH), 1267 (AcO), (Gef.: C, 65·95; H, 8·92; N, 3·29. $C_{24}H_{37}O_5N{\cdot}H_2O$ (M.G. 437·56) Ber.: C, 65·87; H, 8·98; N, 3·20%).

dl-D-*Homo*-18-*nor*-17*α*-*amino*-17*β*-(*β*-*hydroxyäthoxy*)-*androstan*-3*β*-*ol*-13*α*-*carbonsäure*-(13 → 17)-*α*-*laktam* XXIII. Prismen aus An-Ae, Schmp. 250–257°, I.R. ν_{max}^{Nujol} cm^{-1} breite Banden gegen 3500–3100 (OH, NH), 1703 (CO—NH), 1690 (CO—NH verbunden), (Gef.: C, 69·30; H, 9·21; N, 3·48. $C_{22}H_{35}O_4N$ (M.G. 377·51) Ber.: C, 69·99; H, 9·35; N, 3·71%).

C (2) *Degradation von* XXIV *mit* NaOH

Eine Mischung von 123 mg XXIV, 350 mg NaOH und 3·5 cc Äthylenglykol wurde unter einem N_2-Strom und gelegentlicher Schwenkung langsam erhitzt, wobei bei 120° eine Ammoniak-Entwicklung begann. Die innere Reaktionstemperatur wurde 2 Std. bei 120–160° gehalten und dann langsam

bis auf 200° erhöht (1·5 Std.), anschliessend eine halbe Stunde lang auf 200° erhitzt, wobei die NH_3-Entwicklung nur noch sehr schwach war (geprüft mit pH-Indikatorpapier). Nach dem Erkalten wurde mit dem dreifachen Volumen von Wasser versetzt. Nach Entfernung des neutralen Produktes durch Schütteln mit Chf wurde die alkalische Lösung unter Eiskühlung mit konz. HCl bis auf Kongograu angesäuert und dann mit einem Mischlösungsmittel von Chf (3 Vol) und Me (1 Vol) erschöpfend ausgeschüttelt. Die Auszüge ergaben nach Waschen mit der halb gesättigten Na_2SO_4-Lösung, Trocknen über Na_2SO_4 und Eindampfen im Vakuum 115·4 mg saures Produkt, das ohne weiteres mit einer ätherischen Diazomethan-Lösung in Dioxan methyliert wurde. Die übliche Aufarbeitung ergab 107·1 mg Rohprodukt, das an 4 g Al_2O_3 (Woelm Akt. III) chromatographiert wurde. Die mit Bzl–Chf (9:1) eluierten Fraktionen (Nr. 2–4) ergaben aus An und Ae 7·4 mg unbekanntes Produkt (WN 251) vom Schmp. 198–202°, aus der Mutterlauge weitere 9 mg vom Schmp. 165–182°, dessen I.R.-Spektrum ($CHCl_3$) Banden für OH bei 3710, 3638, und 3520 cm^{-1} und für Ester bei 1725 und 1155 cm^{-1} zeigte. Die mit Bzl–Chf (8:2)–(1:1) eluierten Fraktionen (Nr. 9–13) ergaben aus An und Ae 16·8 mg XXV vom Schmp. 222–227°, aus der Mutterlauge noch ein wenig von XXV vom Schmp. 211–218°, dessen I.R.-Spektrum ($CHCl_3$) Banden für OH bei 3700, 3625 und bei 3500 cm^{-1} und für Ester bei 1723 und bei 1142 cm^{-1} zeigte. Die beiden Produkte (WN 251 und XXV) zeigten bei einer Mischprobe mit Ketoester XXI eine deutliche Depression und wurden getrennt einer CrO_3-Oxydation unterworfen (s. unten).

dl-D-*Homo*-18-*nor*-*androstan*-3β,17β-*diol*-13α-*carbonsäuremethylester* XXI. Schmp. 222–227°, I.R. ($CHCl_3$), siehe oben, (Gef.: C, 71·10; H, 9·74. $C_{21}H_{34}O_4$ (M.G. 350·48) Ber.: C, 71·96; H, 9·78%).

WN 251. Schmp. 198–202°, I.R. ($CHCl_3$), siehe oben, (Gef.: C, 68·86; H, 9·20. $C_{21}H_{34}O_5$ (M. G. 366·48) Ber.: C, 68·82; H, 9·35%).

Diese Werte deuten darauf hin, dass dieses Produkt (WN 251) ein Sauerstoff mehr als VI enthält.

C (3) *Oxydation von* XXI *und WN* 251

(i) 13 mg XXI wurden mit CrO_3–Py–Komplex, hergestellt aus 20 mg CrO_3 und 0·2 cc Py, in 0·4 cc Py bei Raumtemperatur über Nacht oxydiert.

Das Reaktionsgut wurde dann in Eiswasser gegossen und dreimal mit Chf extrahiert. Die Chf-Auszüge ergaben nach Waschen der Reihe nach mit 2N H_2SO_4, 2N Na_2Co_3 und mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vakuum ein kristallinisches Produkt, das durch Filtration seiner Lösung in Chf-Bzl (1:1) durch eine Säule von Al_2O_3 (500 mg) gereinigt wurde. Zweimaliges Umkristallisieren ergab 4·2 mg XXV in Platten vom Schmp. 181–183·5°, welches sich in einer Mischprobe und nach dem Vergleich der I.R.-Spektren (in CH_2Cl_2) als identisch mit dem Präparat aus B (4) erwies.

(ii) 12 mg WN 251 wurden analog wie bei (i) mit CrO_3–Py–Komplex oxydiert. Die analoge Aufarbeitung ergab 11·3 mg rohes Produkt, das nach der Reinigung mit Al_2O_3 aus An und Ae umkristallisiert wurde, wobei 2·6 mg Kristalle (WN 253) vom Schmp. 170–173° erhalten wurden. Dieses Produkt zeigte bei der Mischprobe mit XXVI eine deutliche Depression und im I.R.-Spektrum (in CH_2Cl_2) Absorptionen bei 1724 cm^{-1} (Ester), 1708 cm^{-1} (Carbonyl) und bei 1154 cm^{-1} (Ester). Seine Struktur blieb vorläufig unklar und die weitere Untersuchung wurde wegen Materialmangels nicht weiter durchgeführt.

Für die Unterstützung bei dieser Arbeit und die wertvolle Diskussion darüber möchte ich Herrn Doktor K. Takeda, dem Direktor dieses Forschungslaboratoriums sowie Herrn Prof. E. Ochiai herzlichst danken. Ich danke auch Herrn T. Terasawa und Herrn T. Aoki für ihre freundliche Hilfe, Herrn Dr. T. Kubota und Herrn Y. Matsui für die optischen Daten und den Herren vom Mikrolaboratorium für die Analysendaten bestens.

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TOTALSYNTHETISCHE UNTERSUCHUNGEN AN STEROIDEN—VIII*

EINFÜHRUNG DER C-SUBSTITUENTEN IN DIE ANGULARE STELLUNG DER KONDENSIERTEN RINGSYSTEME (TEIL 3). EINWIRKUNG VON KALIUMCYANID UNTER ZUSATZ VON AMMONIUMCHLORID AUF RAC. D-HOMO-18-NOR-ANDROSTANDERIVATE

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Zusammenfassung—Durch Einwirkung von 2 Mol Kaliumcyanid und 1·75 Mol Ammoniumchlorid und nachträgliche Ketalisierung und Acetylierung liess sich rac. D-Homo-18-nor-13(17a)-androstan-3β-ol-17-on (I) in die verschiedenen am C_{13} cyanierten Verbindungen verwandeln. Das Bildungsverhältnis von 13β- zur 13α-Cyanverbindung lag bei 5:3. Die räumlichen Strukturen aller hierbei erhaltenen Verbindungen wurden ermittelt. Die angeführten Reaktionsfolgen stellen eine neue Methode zur Einführung der angularen Substituenten dar und lieferten einen Anhaltspunkt zur Totalsynthese der normalen sowie der in 18-Stellung mit einer funktionellen Gruppe versehenen Steroide.

IN der vorhergehenden Mitteilung[1] dieser Reihe ist eine Methode für die glatte Einführung der Säureamid-Gruppe in die angulare C_{13}-Stellung von rac. D-Homo-18-nor-androst-13(17a)-en-3β-ol-17-on (I) durch Einwirkung von Kaliumcyanid beschrieben worden. Es wurde dabei beobachtet, dass bei der Einwirkung einer begrenzten Menge von KCN auf das Acetat II an Stelle von I und anschliessender Acetylierung, ausser den entsprechenden Laktamol-Verbindungen (VIII, X und XI) noch die epimeren 13β- und 13α-Cyanverbindungen (IV bzw. VI) entstehen. Wegen der Notwendigkeit der präparativen Darstellung der letzteren wurden nun verschiedene Methoden versucht, die eine weitere Verseifung der eingeführten Cyangruppe vermeiden. Die Ergebnisse wurden in Tabelle 1 zusammengestellt.

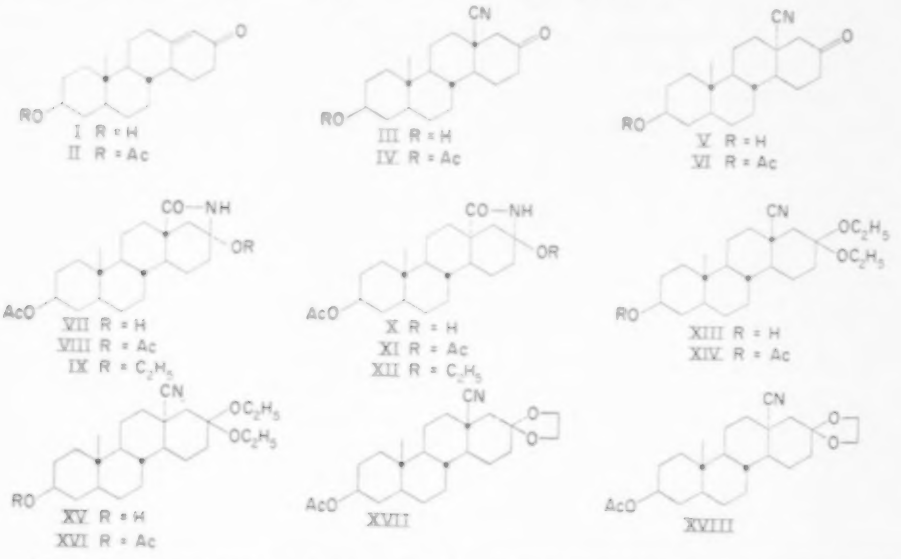
Bekanntlich ist die Addition der freien Blausäure selbst an die Carbonylverbindung wegen ihres geringen Dissoziationsvermögens nicht genügend. Daher wurde sie meistens unter Zusatz von verschiedenen Basen in katalytischer Menge durchgeführt. Die Anwendung dieser Methode bei I erwies sich aber als erfolglos (Versuch 1), wobei Triäthylamin als Base gebraucht wurde. Sogar bei Verwendung der genügenden Menge von Base und unter forcierten Bedingungen verlief die Reaktion nicht glatt. Dabei wurde ausser den erwünschten 13β- und 13α-Cyanverbindungen, IV (16·6%) bzw. VI (1·4%), noch das Acetat des Ausgangsmaterials II in einer Ausbeute von 12·7% zurückgewonnen (Versuch 2). Zweitens wurde Eisessig zur Neutralisierung von KCN in der äquivalenten Menge verwendet, wobei aber die Cyanierung ebenfalls nur in schlechter Ausbeute stattfand (Versuch 3). Aus diesen Tatsachen geht hervor, dass

* VII Mitt.: W. Nagata, *Tetrahedron*, **13**, 268 (1961).

[1] W. Nagata, T. Terasawa, T. Aoki und K. Takeda, VI. Mitt. dieser Reihe, Beitrag zu *Chem. Pharm. Bull. Japan* im Druck.

für die 1,4-Additionsreaktion des Cyanidions an α,β-ungesättigtes Keton, besonders für die Einführung in die angulare Stellung, wegen der kleinen Polarisierbarkeit des letzteren eine hohe Konzentration an Cyanidionen notwendig ist.

Ein Modellversuch,[2] der zu diesem Zweck bei der Cyanierung des Cholestenons durchgeführt worden war, zeigte, dass die Verwendung von 0·75–0·87 Mol. Äquiv. Ammoniumchlorid bezogen auf KCN als Neutralisierungsmittel am besten geeignet ist. Es wurde dabei noch darauf hingewiesen, dass der Vorteil dieses Zusatzes auf folgender Tatsache beruht. Das mit dem Fortgang der Reaktion gebildete Ammoniumion wird durch Erhitzen sofort als Ammoniak-Gas aus der Reaktionslösung vertrieben und ihre Basizität wird damit klein gehalten. Diese Methode wurde nun auf rac. D-Homo-18-nor-13(17a)-androsten-3β-ol (I) angewandt.



Einwirkung von 1·5 Mol Ammoniumchlorid und zwei Mol Kaliumcyanid auf I ergab ein Gemisch von zwei epimeren 13β- und 13α-Cyanverbindungen III und V, das, ohne Trennung der Komponenten, sofort durch Destillation mit abs. Alkohol und Benzol in Gegenwart einer katalytischen Menge *p*-Toluolsulfonsäure ketalisiert wurde. Nach der anschliessenden Acetylierung des Rohproduktes und der Al_2O_3-chromatographischen Trennung wurden 13β- sowie 13α-Cyan-17-diäthylketal-3β-acetat XIV bzw. XVI in einer Ausbeute von 28·7% bzw. 11·2% neben der nicht ketalisierten Verbindung IV (6·7%) erhalten (Versuch 4). Die räumliche Zuordnung der Cyangruppe bei XIV und XVI, nämlich β-Orientierung beim ersteren und α- beim letzteren, wurde durch deren glatte überführung in die entsprechenden Cyanketone IV bzw. VI unter Ketalabspaltung mit wässriger Essigsäure bestätigt, wobei die räumlichen Konfiguration der letzten beiden schon eindeutig festgelegt worden waren.[1] Ein analoger Versuch, wobei Methanol anstatt DMF als Lösungsmittel verwendet wurde, zeigte, dass hierbei ausser den erwünschten Cyanverbindungen noch zwei weiter verseifte, epimere 17-Äthoxylaktamole IX und XII entstanden sind, wenn auch in

[2] W. Nagata, S. Hirai, H. Itazaki und K. Takeda, I. Mitt. unter dem Titel *Über angular substituierte polycyclische Verbindungen* Beitrag zur Zeitschrift *J. Org. Chem.* im Druck.

kleiner Menge (Versuch 5). Dies deutet darauf hin, dass DMF zur Hemmung der weiteren Verseifung der Cyangruppe ein wenig vorteilhafter ist als Methanol. Der Grund dafür dürfte darin zu suchen sein, dass das erstere selbst auch eine Verseifung durch überschüssiges KCN erleiden und somit die Basizität der Lösung vermindern kann.

Noch bessere Ergebnisse konnten durch Verwendung von 0·87 Mol. Äquiv. NH_4Cl bezogen auf KCN verwirklicht werden. α,β-Ungesättigtes Ketol I wurde mit 2M KCN und 1·75M NH_4Cl in siedendem Methanol umgesetzt, wobei eine heftige Ammoniak-Entwicklung beobachtet wurde. Nach der anschliessenden Ketalisierung wie oben konnte man das erhaltene Gemisch von verschiedenen Produkten durch Al_2O_3-Chromatographie leicht trennen. Somit wurden 13β- sowie 13α-Cyandiäthylketal, XIII und XV, und nicht ketalisierte Ketole III und V in einer Gesamtausbeute von 49·6 %[3] erhalten (Versuch 6). 13β-Cyandiäthylketal XIII konnte durch Acetylierung leicht mit dessen Acetat XIV verknüpft werden und das ölige epimere Produkt XV wurde durch Ketalabspaltung mit wässriger Essigsäure in V überführt. Schliesslich wurden die zwei epimeren Ketole III und V acetyliert, deren Acetate sich mit den bekannten Substanzen IV und VI als identisch erwiesen. Auf diese Weise konnten die räumlichen Konfiguration der vier Produkte festgestellt werden. Setzt man nun I ebenfalls mit 2M KCN und 1·75M NH_4Cl in kochendem Methanol um, behandelt das gebildete Produkt üblicherweise mit Äthylenglykol und Benzol in Gegenwart einer katalytischen Menge von *p*-Toluolsulfonsäure und acetyliert das rohe Produkt mit Acetanhydrid und Pyridin, dann kann man nach wiederholtem Chromatographieren zwei epimere 13β- und 13α-Cyanäthylenketalacetate XVII und XVIII neben dem nicht ketalisierten Produkt IV in einer Gesamtausbeute von 65·6 % erhalten (Versuch 7). Da XVII aus IV durch Äthylenketalisierung erhalten wurde, orientiert sich die 13-Cyangruppe des ersteren ebenfalls nach β. Ferner verwandelte sich ein anderes epimeres Produkt XVIII durch Erwärmen mit wässriger Essigsäure leicht in 13α-Cyanketolacetat VI. Wie aus der Tabelle 1 zu ersehen ist, ergab dieser Versuch die beste Ausbeute. Es war hierbei bemerkenswert, dass mehrmaliges Chromatographieren zur Trennung beider epimerer Produkte die gesamte Ausbeute sicher verringerte. 13β-Cyanäthylenketalacetat XVII stellte ein wichtiges Zwischenprodukt zur weiteren Synthese der Steroide dar.[4]

Zum Schluss ergab die Verwendung einer äquimolekularen Menge von NH_4Cl oder NH_4OAc bezogen auf KCN wie aus der Tabelle 1 ersichtlich ist, keine guten Resultate (Versuch 8 und 9).

Für die Orientierung der Richtung bei der Einführung des Cyanidions sollte das Bildungsverhältnis von den 13β- zu den 13α-substituierten Produkten einen Anhaltspunkt geben. Wie aus der Tabelle 1 zu ersehen ist, wich das Verhältnis beim Versuch 7 zwar von demjenigen der anderen drei Versuche (Nr. 4–6) ab, aber das erstere war viel zuverlässiger als das letztere, weil die Reaktion beim Versuch 7 sehr glatt verlief und damit die höchste Gesamtausbeute ergab. Daraus ist zu schliessen, dass die Addition des Cyanidions in diesem Fall[4,5] von der β-Seite des Moleküls im Verhältnis von ca. 5 zu 3 günstiger ist.

[3] Wegen der schwierigen Kristallisierbarkeit wurde das ölige Produkt XV ohne weiteres entketalisiert und dessen Ausbeute wurde, bezogen auf das somit erhaltene 13α-Cyanketol V, berechnet.

[4] s. unsere weiteren Mitteilungen.

[5] Das Bildungsverhältnis der an der angularen Stellung epimeren Cyanverbindungen ist von der Konformation des Ringes oder von der Ringgrösse sehr abhängig.

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TABELLE 1. ANGULARE (C_{13}) CYANIERUNG VON DL-D-HOMO-18-NOR-13(17A)-ANDROSTEN-3β-OL-17-ON (I)

Nr	Mg von I	Umsetzung mit	Menge	Zusatz mit	Menge	Lösgsm.	Reakt., Temp °C	Bedgn. Dauer Std	Nachträgliche Behandlungen	Produkte und (Ausbeute[a], mg)	Total-Ausbeute %	Bildungsverhältnis von 13β- zu 13α subst.-Prodtn.
1	300	HCN	0·2 cc	$(Et)_3N$	3Tropfen	Dioxan	Rt[d]	ü.N.[e]	—	I, (255)		
2	300	HCN	0·3 cc	$(Et)_3N$	0·1 cc	DMF[c]	80–90[f]	5	Ac_2O/Py	II (38·1), TT87 (4·6), VI (4·6), IV (54·3)		
			M.Ä[b]		M.Ä							
3	288	KCN	2·0	HOAc	2·0	EtOH	80–90[f]	6	Ac_2O/Py	VI (27·4), IV (59·7)		
4	300	KCN	2·0	NH_4Cl	1·5	DMF	100	11	(i) EtOH-Bzl/TsOH (ii) Ac_2O/Py	XIV (130·9), XVI (50·8), IV (rohes) (24·8)	46·6	3·16 : 1
5	300	KCN	2·0	NH_4Cl	1·5	CH_3OH	Sieden	8	(i) EtOH/Bzl/TsOH (ii) Ac_2O/Py	XIV (164·1), XVI (21·5), IX (1), XII (27·8)	47·3	3·28 : 1
6	1000	KCN	2·0	NH_4Cl	1·75	CH_3OH	Sieden	6	EtOH-Bzl/TsOH	XV[g] (194·8) → V (72), XIII (345·8), III (132·3), V (62·7)	49·6	3·04 : 1
7	1000	KCN	2·0	NH_4Cl	1·75	CH_3OH	Sieden	6·5	(i) Äthylenglyk. Bzl/TsOH (ii) Ac_2O/Py	XVII (508·8), XVIII (346·5), IV (50·5)	65·6	1·64 : 1
8	300	KCN	2·0	NH_4Cl	2·0	CH_3OH	Sieden	6	EtOH/Bzl/TsOH	XV[g] (76·6) → V (11), XIII (15·7), III (21·7), I (16·7)		
9	300	KCN	2·0	NH_4OAc	2·0	CH_3OH	Sieden	6	EtOH-Bzl/TsOH	XV[g] (44·8) → XV[h] (2·5), XIII (19·6), I (9·5) und (III + V)		

[a] Alle Produkte wurden durch Chromatographie und fraktionierte Kristallisation voneinander getrennt, wobei sich die Schwierigkeiten zur Reinisolierung jedes Stereoisomer, besonders die Trennung des IV von VI, XVII von XVIII und III von V herausstellten.
[b] M.Ä: Mol-Äquivalenz.
[c] DMF: Dimethylformamid.
[d] Rt: Raumtemperatur.
[e] ü.N: über Nacht.
[f] Im Bombenrohr.
[g] XV war sehr schwer zu kristallisieren, und wurde hier nur als Öl erhalten.
[h] XV wurde hierbei in Kristallen erhalten.

BESCHREIBUNG DER VERSUCHE

Alle Schmp. sind auf dem Kofler-Block "Monoskop" (Fa, Hans Bock, Frankfurt/Main, Deutschland) bestimmt und korrigiert worden. Alle Sdp. sind unkorrigiert. Wenn nicht anders angegeben, wurden Substanzproben zur Elementaranalyse 3 Std. bei 1 Torr über P_2O_5 bei Zimmertemperatur bis zu 60°, 70–90° und 100–120° je nach dem Schmp. bis zu 120°, 180° und über 180° getrocknet. Die Adsorptionschromatogramme wurden nach der Durchlaufmethode[6] durchgeführt. Es werden folgende Abkürzungen benützt; Pe = Petroläther, Ae = Äther, Alk = Alkohol, An = Aceton, Bzl = Benzol, Chf = Chloroform, Me = Methanol, Pn = Pentan, Py = Pyridin, Akt. = Aktivität.

(A) *Mit freier Blausäure und Triäthylamin*

Versuch 2 (Tabelle 1). 300 mg (I) wurden in 3 cc Dimethylformamid gelöst, unter Abkühlung mit 0·1 cc $(Et)_3N$ und 0·3 cc frisch hergestellter Blausäure versetzt und dann im Bombenrohr 5 Std. auf 80–90° erhitzt. Das Reaktionsgemisch wurde im Vakuum eingedampft, dreimal mit Chf extrahiert und die Chf-Auszüge wurden mit 2N Na_2CO_3 Lösung, dann mit Wasser gut gewaschen und über Na_2SO_4 getrocknet. Der nach dem Eindampfen gewonne Rückstand wurde mit 2 cc Py und 1·2 cc Ac_2O bei Raumtemperatur über Nacht acetyliert. Nach üblicher Aufarbeitung wurden 423 mg rohes Acetat erhalten, welches sofort an 12 g Al_2O_3 (Woelm Akt. II, neutral) chromatographiert wurde. Die Fraktionen Nr. 6–10 (eluiert mit Pe–Bzl 5:5–3:7) ergaben 38·1 mg unverändertes I-Acetat vom Schmp. 135–137·5° (Mischprobe). Die Fraktionen Nr. 12–17 (eluiert mit Pe–Bzl 3:7–Bzl) ergaben insgesamt 4·6 mg Kristalle vom Schmp. 238–239°, welche I.R.-spektrographisch (Nuj. 2234, 1755, 1727, 1246, 1031 cm^{-1}) die Cyan- und die Lakton-Gruppe enthielten, wurden jedoch nichtn äher untersucht. (Analyse Gef. C, 67·50; H, 7·76). Die mit Bzl–Chf (9:1) eluierten Anteile (die Fraktionen Nr. 19–20) ergaben aus An–Ae 4·6 mg VI[1] vom Schmp. 238–239° (Mischprobe und Vergleich der I.R.-Spektren mit dem authentischen Präparat). Aus den mit Bzl–Chf (7:3 und 5:5) eluierten Fraktionen (Nr. 22–25) wurden aus An–Ae 54·3 mg rohes IV[1] vom Schmp. 205–213° (beim erneuten Umkristallisieren erhöhte sich der Schmp. bis auf 222–226°) erhalten, dessen Identifizierung mit dem authentischen Präparat durch eine Mischprobe und den Vergleich der I.R.-Spektren ausgeführt wurde.

(B) *Mit Kaliumcyanid*

Versuch 3 (Tabelle 1). Zu einer Lösung von 288 mg I und 130 mg KCN in 5 cc 95% Alk und 0·5 cc Wasser wurden 120 mg Eisessig zugegeben und im Bombenrohr 6 Std. auf 80° erhitzt. Der nach gleicher Aufarbeitung wie bei Versuch 2 erhaltene Rückstand wurde ohne weiteres wie üblich bei Raumtemperatur mit Ac_2O und Py acetyliert. Das rohe Acetat 384 mg wurde an 15 g Al_2O_3 (Woelm Akt. III, neutral) chromatographiert, wonach 27·4 mg VI und 59·7 mg IV, erhalten wurden. Die Mischproben derselben mit den authentischen Präparaten zeigten ebenfalls keine Depression.

Versuch 4 (Tabelle 1). Eine Mischung von 300 mg I, 135 mg KCN (2 Mol Äquiv.) und 83 mg (1·5 Mol Äquiv.) NH_4Cl in 5 cc DMF und 1 cc Wasser wurde unter häufiger Schwenkung auf dem Wasserbad bei 100° 11 Std. erhitzt, wobei eine heftige Ammoniak-Entwicklung stattfand. Nach dem Eindampfen im Vakuum wurde der Rückstand mit etwas Wasser versetzt und fünfmal mit Chf–Me (4:1 v/v) extrahiert. Die Auszüge wurden mehrmals mit Wasser gewaschen, über Na_2SO_4 getrocknet und dann im Vakuum eingedampft.

Eine Mischung des so erhaltenen rohen Produkts (356·6 mg), 30 cc abs. Alk und 50 cc abs. Bzl wurde in Gegenwart von 70 mg *p*-Toluolsulfonsäuremonohydrat durch eine Rektifizierungssäule langsam 12 Std. destilliert, wobei das Inhaltvolumen der Reaktionslösung durch ständiges Zutropfen des im gleichen Verhältnis (v/v) zubereiteten Mischlösungsmittels bis auf ca. 1/2–1/3 des anfänglichen beibehalten wurde. 30 mg wasserfreies Natriumacetat wurde zugegeben und dann im Vakuum eingedampft, anschliessend viermal mit Chf ausgeschüttelt. Die Chf-Auszüge wurden zweimal mit Wasser gewaschen, über Na_2SO_4 getrocknet und dann im Vakuum eingedampft.

Das rohe Produkt (412 mg) wurde ohne weiteres mit 2 cc Py und 1·2 cc Ac_2O bei Zimmertemperatur über Nacht acetyliert. Nach üblicher Aufarbeitung wurden 454 mg rohes Acetat erhalten, welches einer Chromatographie an 15 g Al_2O_3 (Woelm Akt. II, neutral) unterworfen wurde.

Die Fraktionen Nr. 2–13 (eluiert mit Pe–Bzl 2:1–1:1) ergaben aus Ae–Pn 130·9 mg dl-D-Homo-18-nor-3β-acetoxy-androstan-17-on-diäthylketal (XIV) als Prismen vom Schmp. 186–190°, 180–186°

[6] T. Reichstein und C. W. Shoppee, *Disc. Faraday. Soc.* Nr. 7, 305 (1949).

je nach der Qualität. Dann folgte aus den Fraktionen Nr. 14–23 (eluiert mit Pe–Bzl 1:1–Bzl) 50·8 mg dl-D-Homo-18-nor-3β-acetoxy-13α-cyan-androstan-17-on-diäthylketal (XVI) als Nadeln (Ae-Pn) vom Schmp. 148–150°. Die mit Bzl–Chf (9:1–8:2) eluierten Anteile (die Fraktionen Nr. 24–27) ergaben 24·8 mg rohes IV vom Schmp. 195–208° (Mischprobe) und zuletzt wurde aus den Fraktionen Nr. 35–38 (Chf) ein wenig Dimeres (Schmp. über 300°) erhalten. Die Hauptprodukte XIV und XVI wurden zur Analyse weiter gereinigt und haben folgende Analysen- sowie physikalischen Werte.

dl-D-*Homo*-18-*nor*-3β-*acetoxy*-13β-*cyan-androstan*-17-*on-diäthylketal* XIV. Prismen vom Schmp. 186–190° aus Ae–Pn, I.R. ν_{max}^{Nujol} cm^{-1} 2215 (CN), 1741 (AcO—), 1241 (AcO—), 1126, 1057 $\left(<\begin{matrix}OEt\\OEt\end{matrix}\right)$, 1028 (AcO—). (Gef.: C, 72·39; H, 9·59; N, 3·20. $C_{26}H_{41}O_4N$ (M.G. 431·60) Ber.: C, 72·35; H, 9·58; N, 3·25%).

dl-D-*Homo*-18-*nor*-3β-*acetoxy*-13α-*cyan-androstan*-17-*on-diäthylketal* XVI. Nadeln vom Schmp. 148–150° aus Ae–Pn, I.R. ν_{max}^{Nujol} cm^{-1} 2223 (CN), 1740 (AcO—), 1239 (AcO—), 1135, 1060 $\left(<\begin{matrix}OEt\\OEt\end{matrix}\right)$, 1027 (AcO—). (Gef.: C, 72·21; H, 9·52; N, 3·26. $C_{26}H_{41}O_4N$ (M.G. 431·60) Ber.: C, 72·35; H, 9·58; N, 3·25%).

Versuch 5 (Tabelle 1). 300 mg I wurden in 5 cc Me gelöst, mit 135 mg (2·0 Mol Äquiv.) KCN, 83 mg (1·5 Mol Äquiv.) NH_4Cl in 1 cc Wasser versetzt und dann 8 Std. zum Sieden erhitzt, wobei gleichfalls eine heftige NH_3-Entwicklung stattfand. Nach analoger Aufarbeitung wie bei Versuch 4 wurden 371·6 mg rohes Produkt erhalten, welches mit Bzl und Alk in Gegenwart von TsOH in gleicher Weise ketalisiert wurde. Das rohe Diäthylketal (392 mg) wurde sofort mit 2 cc Py und 1·2 cc Acetanhydrid bei Raumtemperatur über Nacht acetyliert. Nach üblicher Aufarbeitung wurden 438 mg rohes Acetat erhalten, welches an 15 g Al_2O_3 (Woelm Akt. II, neutral) chromatographiert wurde. Daraus wurden gleicherweise wie bei Versuch 4, 164 mg rohes XIV (aus den mit Pe–Bzl 8:2–1:2 eluierten Fraktionen), 21·5 mg XVI (aus den mit Pe–Bzl 1:2–Bzl eluierten Fraktionen) 1 mg β-Laktam-Verbindung IX vom Schmp. 262–263° (Mischprobe) (aus den mit Bzl–Chf 8:2 eluierten Fraktionen) und 27 mg α-Laktam-Verbindung XII vom Schmp. 206–207° (Mischprobe) (aus den mit Bzl–Chf 1:1 eluierten Fraktionen) erhalten.

Versuch 6 (Tabelle 1). 1 g I wurde in 16 cc Me gelöst. Dazu wurde eine Lösung von 450 mg KCN (6·92 mili-Mol) und 325 mg NH_4Cl (6·07 mili-Mol) in 3 cc Wasser zugegeben und dann auf dem Wasserbad 6 Std. unter Rückfluss gekocht. Das nach gleicher Aufarbeitung erhaltene rohe Produkt (1·15 g) wurde mit einer Mischung von 100 cc abs. Alk und 165 cc abs. Bzl analog wie beim Versuch 4 ketalisiert und aufgearbeitet. Das rohe Diäthylketal (1·328 g) wurde ebenfalls an 30 g Al_2O_3 (Merck, Akt. II) chromatographiert. Die mit Bzl, Bzl–Chf (95:5) und Bzl–Chf (90:10) eluierten Anteile (die Fraktionen Nr. 2–16) ergaben insgesamt 277·4 mg rohes dl-D-Homo-18-nor-13β-cyan-androstan-3β-ol-17-on-diäthylketal XIII aus An–Ae als dünne Platten vom Schmp. 173–176°, 174–177° und 174–176°. Die Mutterlaugen aus diesen Fraktionen wurden vereinigt und erneut chromatographiert (s. unten). Die mit Bzl–Chf (75:25 und 50:50) eluierten Anteile (die Fraktionen Nr. 18–24) ergaben zuerst insgesamt 132·3 mg rohes dl-D-Homo-18-nor-13β-cyanandrostan-3β-ol-17-on III aus An–Ae oder nur An als dünne Platten vom Schmp. 234–238° und 233–241° und aus Mutterlaugen durch wiederholte Umkristallisation insgesamt 62·7 mg rohes dl-D-Homo-18-nor-13α-cyanandrostan-3β-ol-17-on V vom Schmp. 238–244° und 236–242°, welche beide durch fraktionierte Kristallisationen aus An oder An–Ae rein getrennt wurden. Zuletzt wurde aus den Fraktionen Nr. 27–30 (eluiert mit Chf) ein wenig Kristallgemisch vom Schmp. 190–225° erhalten, das nicht weiter untersucht wurde.

Die Mutterlaugen aus den Fraktionen Nr. 1–16 wurden vereinigt (578·7 mg) und erneut an 15 g Al_2O_3 chromatographiert. Die mit Pe–Bzl (5:5) eluierten Anteile (die Fraktionen Nr. 6–13) ergaben 194·8 mg öliges[7] dl-D-Homo-18-nor-13α-cyan-androstan-3β-ol-17-on-diäthylketal XV, das durch Einwirkung von verdünnter Essigsäure in die bekannte 13α-Cyan-Verbindung V übergeführt wurde (s. unten). Die mit Pe–Bzl (3:7), Bzl und Bzl–Chf (95:5, 90:10 und 70:30) eluierten Anteile ergaben weitere 64·8 mg von XIII. Aus beiden Chromatographien wurden dann 194·8 mg öliges XV, 345·8 mg XIII, 132.3 mg III und 62·7 mg V der Reihe nach, je nach der Eluierungsfähigkeit erhalten.

Die Produkte XIII, III und V wurden zur Analyse weiter gereinigt und haben folgende physikalische sowie Analysen-Werte.

[7] Im Versuch 9 wurde ein Teil dieses Produktes in kristallinischer Form erhalten. s. unten.

dl-D-*Homo*-18-*nor*-13*β*-*cyan*-*androstan*-3*β*-*ol*-17-*on*-*diäthylketal* XIII. Feine dünne Platten vom Schmp. 177–179° aus An-Ae, I.R. ν_{max}^{Nujol} cm^{-1} 3549, 3285 (OH), 2229 (CN), 1130, 1058, 974 (<OEt/OEt). (Gef.: C, 72·95; H, 10·22; N, 3·48. $C_{24}H_{39}O_3N \cdot 1/3H_2O$ (M.G. 395·56) Ber.: C, 72·87; H, 10·10; N, 3·54%).

dl-D-*Homo*-18-*nor*-13*β*-*cyan*-*androstan*-3*β*-*ol*-17-*on* III. Dünne Platten vom Schmp. 239–242° aus An-Ae, I.R. ν_{max}^{Nujol} cm^{-1} 3505 (OH), 2232 (CN), 1703 (C=O). (Gef.: C, 76·18; H, 9·42; N, 4·51. $C_{22}H_{33}O_2N$ (M.G. 315·44) Ber.: C, 76·15; H, 9·27; N, 4·44%).

dl-D-*Homo*-18-*nor*-13*α*-*cyan*-*androstan*-3*β*-*ol*-17-*on* V. Prismen vom Schmp. 244–246° aus An-Ae, I.R. ν_{max}^{Nujol} cm^{-1} 3496 (OH), 2228 (CN), 1704 (C=O). (Gef.: C, 75·97; H, 9·51; N, 4·64. $C_{22}H_{33}O_2N$ (M.G. 315·44) Ber.: C, 76·15; H, 9·27; N, 4·44%).

Entketalisierung von dl-D-*Homo*-18-*nor*-13*α*-*cyan*-*androstan*-3*β*-*ol*-17-*on*-*diäthylketal* XI *mit verdünnter Essigsäure*. 194·8 mg von dem oben beschriebenen öligen XV wurden in 2·5 cc Eisessig gelöst, in der Wärme mit 1·7 cc Wasser versetzt und dann 20 Min. auf 80° erhitzt. Die Essigsäure wurde im Vakuum abdestilliert, der Rückstand dreimal mit Chf extrahiert und die Chf-Auszüge wurden mit 2N Na_2CO_3 und mit Wasser gewaschen und dann über Na_2SO_4 getrocknet. Das Eindampfen des Chf im Vakuum hinterliess 165 mg kristallinischen Rückstand, welcher aus An–Ae 72·0 mg Kristalle vom Schmp. 229–235° ergab. Die weitere Reinigung derselben wurde durch nochmalige Umkristallisation bewirkt. Der Schmp. und Mischschmp. mit authentischem V waren 233–241° und beide I.R.-Spektren in Nujol waren identisch, Mischprobe mit III zeigte merkliche Depression.

Acetylierung von dl-D-*Homo*-18-*nor*-13*β*-*cyan*-*androstan*-3*β*-*ol*-17-*on* (III) 83 mg III wurden mit 0·9 cc Ac_2O und 1·5 cc Py bei Zimmertemperatur über Nacht acetyliert. Die übliche Aufarbeitung ergab 94·5 mg kristallinisches rohes Acetat, welches durch Chromatographie an Al_2O_3 gereinigt wurde. Aus den mit Bzl und Bzl–Chf (9:1) eluierten Fraktionen wurden 37·6 mg IV vom Schmp. 224–227° erhalten. Mischprobe mit dem authentischen Präparat von IV zeigte keine Depression.

Acetylierung von dl-D-*Homo*-18-*nor*-13*α*-*cyan*-*androstan*-3*β*-*ol*-17-*on* (V). 23·5 mg V wurden mit 0·6 cc Ac_2O und 1 cc Py bei Zimmertemperatur über Nacht acetyliert. Die übliche Aufarbeitung ergab aus An–Ae 9·8 mg Kristalle vom Schmp. 235–237° und aus der Mutterlauge 3·0 mg vom Schmp. 234–240°. Mischprobe mit dem authentischen Präparat von VI zeigte keine Depression.

Versuch 7 (Tabelle 1). 1 g I wurde analog wie bei dem Versuch 6 mit 450 mg KCN und 325 mg NH_4Cl in siedendem Me (16 cc) und Wasser (4 cc) 6·5 Std. gekocht. Die gleiche Aufarbeitung ergab 1·276 g schaumförmiges rohes Produkt, das in 150 cc abs. Bzl in Gegenwart von 50 mg *p*-Toluolsulfonsäuremonohydrat in üblicher Weise mit 1 cc Äthylenglykol ketalisiert wurde, wobei es bis zum vollständigen Verschwinden der Trübung des Destillates etwa 8·5 Std. dauerte. Es wurde dann mit etwas Wasser versetzt und die organische von der wässrigen Phase getrennt. Die letztere wurde dreimal mit Chf extrahiert. Die organischen Phasen wurden mit 2N Na_2CO_3 und mit Wasser gewaschen, über Na_2SO_4 getrocknet und dann im Vakuum eingedampft. Der Eindampfrückstand (1·433 g) wurde ohne weiteres mit 6 cc Ac_2O und 10 cc Py bei Zimmertemperatur acetyliert. Das erhaltene rohe Acetatgemisch (1·458 g) wurde durch viermaliges Chromatographieren an Al_2O_3 und fraktioniertes Umkristallisieren in die drei Hauptbestandteile getrennt. Aus der ersten Chromatographie (60 g Al_2O_3, Woelm Akt. II, neutral) wurden aus An–Ae insgesamt 284·0 mg dl-D-Homo-18-nor-3*β*-acetoxy-13*β*-cyan-androstan-17-on-äthylenketal XVII als Säulen vom Schmp. 235–239°, 237–240° und 236–237° je nach der Qualität (aus den Fraktionen Nr. 6–18, eluiert mit Pe–Bzl 7:3–Pe-Bzl 5:5) und 50·5 mg dl-D-Homo-18-nor-3*β*-acetoxy-13*β*-cyan-androstan-17-on IV vom Schmp. 220–225° (Mischprobe, aus den Fraktionen Nr. 33–36, eluiert mit Bzl) erhalten.

Die Mutterlaugen sowie das Kristallgemisch aus den Fraktionen Nr. 6–18 und Nr. 33–36, und die Fraktionen Nr. 19–32 wurden vereinigt (870 mg) und einer zweiten Chromatographie (30 g Al_2O_3) unterworfen, woraus weitere 145·4 mg von XVII vom Schmp. 236–238° und 233–240° aus den Fraktionen Nr. 3–11 (eluiert mit Pe–Bzl 8:2) und dann aus An-Ae insgesamt 78·6 mg dl-D-Homo-18-nor-3*β*-acetoxy-13*α*-cyan-androstan-17-on-äthylenketal XVIII als Platten oder Prismen vom Schmp. 220–226° und Schmp. 215–226° (aus den Fraktionen Nr. 22–30, eluiert mit Pe–Bzl 6:4–5:5) erhalten wurden. Die übrige Anteile wurden vereinigt (620 mg) und einer dritten Chromatographie (15 g Al_2O_3) unterworfen, woraus ebenfalls 69·2 mg XVII und 232·7 mg XVIII erhalten wurden. Aus der vierten Chromatographie (8 g Al_2O_3) wurden weitere 10·2 mg XVII und 35·2 mg XVIII erhalten. Aus den vier Chromatographien wurden dann total 508·8 mg XVII, 346·5 mg XVIII und 50·5 mg

IV herausgezogen und die zwei isomeren Hauptprodukte (XVII und XVIII) wurden weiter gereinigt und wie folgt identifiziert.

dl-D-*Homo*-18-*nor*-3β-*acetoxy*-13β-*cyan-androstan*-17-*on-äthylenketal* XVII. Säulen aus An–Ae, Schmp. 233–235° oder dicke Prismen aus Chf–Ae Schmp. 235–238°, I.R. ν_{max}^{Nujol} cm^{-1} 2233 (CN), 1730 (AcO—), 1242, 1025 (AcO—), 1103, 953 $\left(<\!\!\begin{matrix}O\\O\end{matrix}\!\!\right]$). (Gef.: C, 71·88; H, 8·73; N, 3·51. $C_{24}H_{35}O_4N$ (M.G. 401·53) Ber.: C, 71·79; H, 8·79; N, 3·49%).

dl-D-*Homo*-18-*nor*-3β-*acetoxy*-13α-*cyan-androstan*-17-*on-äthylenketal* XVIII. Platten aus An-Ae, Schmp. 224–226°, I.R. ν_{max}^{Nujol} cm^{-1} 2246 (CN), 1725 (AcO—), 1239, 1030 (AcO—), 1103 $\left(<\!\!\begin{matrix}O\\O\end{matrix}\!\!\right]$). (Gef.: C, 71·46; H, 8·80; N, 3·32. $C_{24}H_{35}O_4N$ (M.G. 401·53) Ber.: C, 71·79; H, 8·79; N, 3·49%).

Äthylenketalisierung von dl-D-*Homo*-18-*nor*-13β-*cyan*-3β-*acetoxy-androstan*-17-*on* (IV). 660 mg IV wurden in 100 cc abs. Bzl in Gegenwart von 50 mg *p*-Toluolsulfonsäuremonohydrat mit 1 cc Äthylenglykol wie üblich ketalisiert. Die übliche Aufarbeitung ergab 861 mg kristallinisches rohes Produkt, das sofort mit 4 cc Ac_2O und 7 cc Py bei Zimmertemperatur zurückacetyliert wurde.

Das nach der üblichen Aufarbeitung gewonnene rohe Acetat (858 mg) wurde aus Chf–An umkristallisiert und ergab 547 mg dicke Prismen vom Schmp. 235–238° (weitere 94 mg vom Schmp. 223–229° aus der Mutterlauge), die sich nach einer Mischprobe sowie dem Vergleich der I.R.-Spektren mit XVII als identisch erwiesen.

Entketalisierung von dl-D-*Homo*-18-*nor*-13α-*cyan*-3β-*acetoxy-androstan*-17-*on-äthylenketal* (XVIII). 130 mg XVIII wurden in 2·8 cc Eisessig gelöst, in der Wärme mit 1·2 cc Wasser versetzt und dann 20 Min. auf 80° erhitzt. Im Vakuum wurde die Lösung eingedampft, dreimal mit Chf extrahiert und die Chf-Auszüge mit 2N Na_2CO_3, dann mit Wasser gewaschen, über Na_2SO_4 getrocknet und im Vakuum eingedampft. Der kristallinische Rückstand (116 mg) ergab bei der Umkristallisation aus An–Ae 80·5 mg lange Platten vom Schmp. 237–239°, und aus der Mutterlauge weitere 23·5 mg vom Schmp. 234–239°. Die Mischprobe mit authentischem VI (Schmp. 239–241°) zeigte keine Depression.

Versuch 8 (Tabelle 1). 300 mg I wurden in 5 cc Me gelöst, wozu eine Lösung von 135 mg (2 Mol Äquiv.) KCN und 111 mg (2 Mol Äquiv.) NH_4Cl in 1 cc Wasser zugegeben und dann 6 Std. zum Sieden erhitzt wurde. Die Aufarbeitung wie bei Versuch 6 ergab 377 mg rohes Produkt, welches sofort mit einem Gemisch von abs. Bzl und abs. Alk (5:3) in Gegenwart von 70 mg *p*-Toluolsulfonsäuremonohydrat ketalisiert wurde. Nach 17 stündiger Destillation wurde die Reaktionslösung ebenso wie bei Versuch 6 aufgearbeitet. Der rohe Rückstand (403·2 mg) wurde an 15 g Al_2O_3 (Merck, Akt. II) chromatographiert. Die mit Bzl eluierten Anteile (die Fraktionen Nr. 9–13) ergaben 76·6 mg öliges XV, welches beim Erhitzen mit 70% wässriger Essigsäure 11 mg V vom Schmp. 227–232° ergab. Die Identität desselben mit dem authentischen Präparat wurde durch eine Mischprobe und den Vergleich der I.R.-Spektren erzielt. Danach folgten mit dem gleichen Lösungsmittel 15·7 mg X vom Schmp. 173–175° (Mischprobe; aus den Fraktionen Nr. 14–23) und dann 16·7 mg Ausgangsmaterial (I) vom Schmp. 182–185° (Mischprobe; aus den Fraktionen Nr. 26–29). Zuletzt wurden aus den Fraktionen Nr. 37–44 (Bzl–Chf 8:2–1:1) 21·7 mg nicht ketalisiertes III vom Schmp. 238–245° (Mischprobe) erhalten.

Versuch 9 (Tabelle 1). Eine Lösung von 300 mg I, 135 mg (2 Mol Äquiv.) KCN und 160·2 mg (2 Mol Äquiv.) NH_4OAc in 5 cc Me und 1 cc Wasser wurde 6 Std. unter Rückfluss gekocht. Die gleiche Aufarbeitung ergab 352·1 mg rohes Produkt, welches sofort analog wie bei Versuch 8 diäthylketalisiert wurde. Nach analoger Aufarbeitung wurden 378 mg rohes Produkt erhalten, das an 15 g Al_2O_3 chromatographiert wurde. Die mit Bzl eluierten Anteile (die Fraktionen Nr. 3–5) ergaben ebenfalls 44·8 mg öliges XV, welches beim Ansetzen mit Ae und Pn 2·5 mg Nadeln vom Schmp. 110–112° ergab. Diese Kristalle waren offenbar dl-D-Homo-18-nor-13α-cyanandrostan-3β-ol-17-on-diäthylketal XV, welches aber wegen Materialmangels nicht weiter untersucht wurde. Der ölige Anteil (40 mg) ergab beim kurzen Erhitzen mit 70% Essigsäure auf dem Wasserbad ebenfalls 4.0 mg V aus An–Ae (Mischprobe). Dann wurde aus den Fraktionen Nr. 6–10 (eluiert mit Bzl) 19·6 mg XIII (Mischprobe), aus den Fraktionen Nr. 14–19 (eluiert mit Bzl–Chf 9:1) 9·5 mg Ausgangsmaterial (Mischprobe) und etwas Kristallgemisch von III und V (gemäss dem I.R.-Spektrum) erhalten.

Acetylierung von dl-D-*Homo*-18-*nor*-13β-*cyan-androstan*-3β-*ol*-17-*on-diäthylketal* (XIII). 35·7 mg XIII wurden wie üblich mit 0·3 cc Ac_2O und 0·5 cc Py bei Zimmertemperatur über Nacht acetyliert. Die übliche Aufarbeitung ergab 43·9 mg rohes Acetat, welches aus An–Ae 29 mg Platten vom Schmp. 183–185° ergab. Mischprobe mit dem authentischen Präparat von XIV zeigte keine Depression.

Entketalisierung von dl-D-*Homo*-18-*nor*-3β-*acetoxy*-13β-*cyan-androstan*-17-*on-diäthylketal* (XIV) und *seinem* 13α-*Isomer* (XVI). 100 mg XIV wurden in 2 cc Eisessig gelöst, in der Wärme mit 1·5 cc Wasser versetzt und dann 1 Std. auf 80° erhitzt. Die Essigsäure wurde im Vakuum eingedampft, der Rückstand dreimal mit Chf extrahiert und die Chf-Auszüge mit 2N Na_2CO_3 und Wasser gewaschen, über Na_2SO_4 getrocknet und dann im Vakuum eingedampft. Der kristallinische Rückstand (85·7 mg) ergab aus An–Ae 67·6 mg Prismen vom Schmp. 219–221° und aus der Mutterlauge weitere 9·1 mg vom Schmp. 205–213°. Diese Kristalle zeigten bei der Mischprobe mit dem authentischen Präparat von IV (Schmp. 221–225°) keine Depression.

40 mg XVIII wurden ebenfalls mit 0·8 cc 70% Essigsäure deketalysiert und ergaben 26·7 mg VI vom Schmp. 229–234° (Mischprobe).

Für die Unterstützung bei dieser Arbeit und die wertvolle Diskussion darüber möchte ich Herrn Doktor K. Takeda, dem Direktor dieses Forschungslaboratoriums sowie Herrn Prof. E. Ochiai herzlichst danken. Ich danke auch Herrn T. Terasawa und Herrn T. Aoki für ihre freundliche Hilfe, Herrn Dr. T. Kubota und Herrn Y. Matsui für die optischen Daten und den Herren vom Mikrolaboratorium für die Analysendaten bestens.

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TOTALSYNTHETISCHE UNTERSUCHUNGEN AN STEROIDEN—IX*

EINFÜHRUNG DER C-SUBSTITUENTEN IN DIE ANGULARE STELLUNG DER KONDENSIERTEN RINGSYSTEME (TEIL 4). DARSTELLUNG VON RAC. D-HOMO-18-OXO-ANDROSTAN-3β-OL-17-ON

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Zusammenfassung—Die Reduktion von rac, D-Homo-18-nor-13β-cyan-androstan-3β-ol-17-on-3-acetat-17-äthylenketal IV mit einem Überschuss an $LiAlH_4$ in Tetrahydrofuran bei Raumtemperatur ergab nach kurzem Kochen mit Natronlauge unter Stickstoffatmosphäre und nach Zurückacetylierung das 18-Oxo-derivat XV in guter Ausbeute, das sich durch Huang Minlon'sche Reduktion und nachfolgende Ketalabspaltung und Acetylierung glatt in rac. D-Homo-androstan-3β-ol-17-on-3-acetat XVIII überführen liess. Diese Reduktionsprozesse stellten eine neue Methode für die Einführung der angularen C-Substituenten dar.

In der vorangehenden Mitteilung* dieser Reihe ist eine Methode zur Darstellung von 13β- sowie 13α-Cyanderivaten der rac. D-Homoandrostan-Reihe, z.B. II, III, IV und V, bevorzugt an den β-Epimeren beschrieben worden, die in der Einwirkung von Kaliumcyanid in Gegenwart von Ammoniumchlorid auf rac. D-Homo-18-nor-13(17a)-androsten-3β-ol-17-on (I) besteht. In dieser Mitteilung sind die Ergebnisse der Reduktion dieser angular stehenden Cyangruppe beschrieben.

Der Modellversuch[1] zeigte, dass 5α-Cyancholestan-3-on-3-äthylenketal VI durch Einwirkung von überschüssigem $LiAl(OEt)_3H$[2] oder $LiAlH_4$, besser mit dem letzteren Reagenz, sehr glatt zum Imid VIII reduziert wird, das sich bei der Behandlung mit Aluminiumoxyd leicht in Aldehyd IX verwandelt. 5α-Aldehyd IX konnte dann durch Huang Minlon'sche Reduktion in 5α-Methyl-cholestan-3-on-3-äthylenketal X in einer Ausbeute von über 90% reduziert werden. Im Gegensatz zum 5α-Cyankörper ergab 5β-Cyan-cholestan-3-on-3-äthylenketal VII bei der Reduktion sowohl mit $LiAl(OEt)_3H$ und $LiAl(OEt)_2H_2$,[3] als auch mit $LiAlH_4$ immer ein Gemisch von Aldehyd XI, Amin XIII und Anil XII, was darauf hindeutete, dass die Reduktion hierbei nicht auf der Imid-Stufe stehenbleibt.

Gemäss den Ergebnissen dieses Modellversuches wurde 13β-Cyan-17-äthylenketal IV einer Reduktion mit Lithiumalkoxyaluminium- oder Lithiumaluminium-Hydrid in wasserfreiem Tetrahydrofuran (THF) unterworfen. Zuerst erwies sich die Verwendung von $LiAl(OEt)_2H_2$[3] als etwas besser, wobei die erwünschte 18-Oxoverbindung XV nach kurzem Kochen des rohen Reduktionsproduktes mit der verd. Natronlauge neben dem Ausgangsmaterial in einer Ausbeute von 21% erhalten wurde. Das beste

* VIII Mitt: W. Nagata, *Tetrahedron* **13**, 278 (1961).

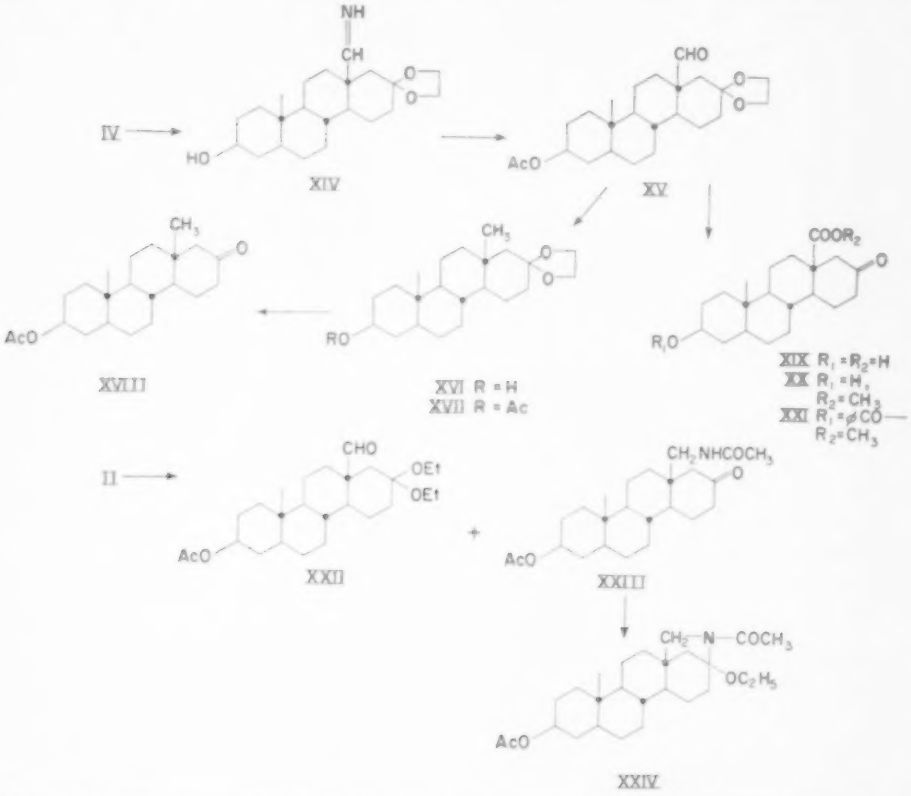
[1] W. Nagata, S. Hirai, H. Itazaki und K. Takeda, VI. Mitt. unter dem Titel *Über die angular substituierten polycyclischen Verbindungen* Beitrag zu *Liebigs Ann.* im Druck.
[2] H. C. Brown, C. J. Shoaf und C. P. Garg, *Tetrahedron Letters* No. 3, 9 (1959).
[3] H. C. Brown und R. F. MacFarlin, *J. Amer. Chem. Soc.* **81**, 502 (1959).

Resultat konnte aber durch Reduktion mit überschüssigem $LiAlH_4$ erzielt werden. IV wurde mit einem Überschuss an $LiAlH_4$ in THF bei Raumtemperatur reduziert. Nach der Aufarbeitung wurde XIV in kristallinischer Form erhalten. Es zeigte in seinem I.R.-Spektrum zwei scharfe Banden für Imid bei 3270 cm^{-1} (=NH) und bei 1633 cm^{-1} (—CH=NH). Es wurde, ohne es zu isolieren, durch kurzes Kochen unter Stickstoff mit 2N Natronlauge in Methanollösung zum Aldehyd verseift. Der nach Zurückacetylierung in guter Ausbeute erhaltene Aldehyd XV zeigte in seinem I.R.-Spektrum zwei deutliche CH-Schwingungsbanden des Aldehydes bei 2742 cm^{-1} und 2680 cm^{-1} neben der Aldehyd-Carbonylbande, und im U.V.-Spektrum ein Maximum bei 290–292 mμ ($\varepsilon = 35$). Die Analysenwerte stimmten mit der Formel XV gut überein. Zur weiteren Bestätigung wurde XV in den bekannten Ketocarbonsäure-methylester[4] XX übergeführt. XV wurde dazu mit CrO_3 und H_2SO_4 in Aceton[5] oxydiert und das erhaltene Produkt, ohne zu isolieren, durch eine Reihe von Operationen, viz. Entketalisierung mit verd. Salzsäure, Verseifung mit verd. Na_2CO_3 und anschliessender Methylierung der freien Carbonsäure XIX mit Diazomethan in einen Methylester übergeführt, dessen Identität mit dem authentischen Ester XX durch eine Mischprobe, sowie direkten Vergleich der I.R.-Spektren sowohl des Esters selbst als auch von dessen Benzoat XXI festgestellt wurde. XV wurde andererseits nach Huang Minlon reduziert und das in guter Ausbeute erhaltene 18-Deoxo-Derivat XVI

[4] VII Mitt.: W. Nagata, *Tetrahedron* **13**, 268 (1961).
[5] A. Bowers, T. G. Halsall, E. R. H. Jones, A. J. Lemin, *J. Chem. Soc.* 2548 (1953).

acetyliert. Die Ketalabspaltung des Acetates XVII mit wässriger Essigsäure ergab rac. D-Homo-androstan-3β-ol-17-on Acetat XVIII. Für präparative Zwecke erwies sich ein Durchlauf-Verfahren als vortrefflich. Dabei wurde von der 13β-Cyanverbindung IV ausgehend, ohne Isolierung der Zwischenprodukte das Endprodukt XVIII in einer Ausbeute von 73% erhalten.

Als nächstes wurde das Verhalten von 13β-Cyan-17-diäthylketal II gegenüber Lithiumalkoxyaluminium- sowie Lithiumaluminium-Hydrid untersucht. Gegen alle Erwartungen verhielt sich II sowohl gegen das erste Reagenz als auch gegen das letztere ähnlich wie 5β-Cyan-cholestan-3-on-3-äthylenketal VII. Obwohl II in der



Kälte fast nicht angegriffen wird, ergab es bei der Einwirkung von $LiAlH_4$ bei Raumtemperatur ein Gemisch von einem primären Amin und einem Imid, wobei das Amin bevorzugt gebildet wurde. Durch nachträgliche Verseifung und Acetylierung des Reduktionsproduktes wurde nun neben einer in geringer Menge gebildeten Substanz vom Schmp. 143–147° eine Substanz vom Schmp. 233–236° erhalten. Gemäss ihrem I.R.-Spektrum (Aldehydbanden bei 2710 cm^{-1} und 1718 cm^{-1}) und den Analysenwerten konnte man gut erkennen, dass die erstere den erwünschten Aldehyd XXII darstellt. Die zweite Substanz zeigte in ihrem I.R.-Spektrum Banden für Imid bei 3338 cm^{-1}, für Acetat bei 1734 cm^{-1}, für 6-gliedriges Ringketon bei 1715 cm^{-1} und für primäres Amid bei 1685 cm^{-1} und bei 1534 cm^{-1} und wies eine positive Zimmermann-Farbreaktion auf, was mit der Formel XXIII gut vereinbar war. Die Bildung

des freien 17-Ketons beruht scheinbar auf der Einwirkung von Weinsäure, die zur Zersetzung des Reaktionsgutes verwendet worden war. Zur Sicherung dieser Struktur wurde XXIII mit abs. Alkohol und abs. Benzol in Gegenwart von *p*-Toluolsulfonsäure langsam destilliert. Nach Zurückacetylierung des Rohproduktes wurde ein Produkt vom Schmp. 179–180° erhalten, dessen I.R.-Spektrum keine Banden für Ringketon, für Imid und für primäres oder nicht cyclisches Säureamid mehr zeigte. Demgemäss dürfte man diesem die Formel XXIV zukommen lassen.

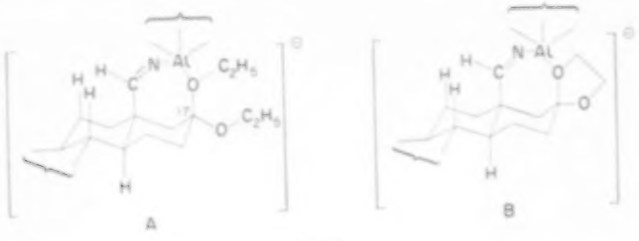


ABB. 1.

Den Grund, warum die Reduktion von 13β-Cyan-diäthylketal II mit $LiAlH_4$ nicht auf der Imid-Stufe stehen bleibt, könnte man in der Instabilität des intermediären Al-Komplexanions* A im Vergleich zu demjenigen (B) des 17-Äthylenketals IV wegen der Drehbarkeit und der Biegsamkeit der C_{17}-O Bindung finden.

BESCHREIBUNG DER VERSUCHE

Alle Schmp. sind auf dem Kofler-Block "Monoskop" (Fa. Hans Bock, Frankfurt/Main, Deutschland) bestimmt worden und sind korrigiert. Die Sdp. sind alle unkorrigiert. Wenn nicht anders angegeben, wurden Substanzproben zur Elementaranalyse 3 Std. lang bei 1 Torr über P_2O_5 bei Zimmertemperatur bis 60°, 70–90° und 100–120° je nach dem Schmp. von bis 120°, 180° und mehr getrocknet. Die Adsorptionschromatogramme wurden nach der Durchlaufmethode[6] durchgeführt. Es werden folgende Abkürzungen benützt; Pe = Petroläther, Ae = Äther, Alk = Alkohol, An = Aceton, Bzl = Benzol, Chf = Chloroform, Me = Methanol, Pn = Pentan, Py = Pyridin, Akt. = Aktivität.

1. *Reduktion von* 13β*-Cyan-*17*-diäthylketal*

(1) Reduktion von dl-D-Homo-18-nor-13β-cyan-androstan-3β-ol-17-on-3-acetat-17-diäthylketal (II) mit $LiAlH_4$. 200 mg II wurden in 9 cc abs. Tetrahydrofuran (THF) gelöst. Dazu wurde eine Lösung von 176 mg $LiAlH_4$ in 15 cc abs. THF unter Rühren und Eis-Kühlung innerhalb von 30 Min. getropft und dann bei Raumtemperatur 3 Std. lang weiter gerührt. Die Reaktionslösung wurde unter Eiskühlung mit einem Gemisch von 10 cc einer 0·886M Rochelle'schen Salz-Lösung und 2 cc einer 0·5M Weinsäurelösung versetzt und nach Zugabe von etwas Wasser mit Chf extrahiert. Die Chf-Auszüge wurden nach Waschen mit Wasser (zweimal) und Trocknen über Na_2SO_4 im Vakuum eingedampft. Der Rückstand (189·6 mg) wurde dann in 14·5 cc Me gelöst, mit 5·8 cc 2N NaOH versetzt und unter einem N_2-Strom 5 Min. unter Rückfluss gekocht. Die übliche Aufarbeitung ergab 203 mg Eindampfrückstand, der ohne weiteres mit 2·4 cc Ac_2O und 3 cc Py bei Raumtemperatur über Nacht acetyliert wurde. Die übliche Aufarbeitung ergab 248 mg teilweise kristallisierten Eindampfrückstand. Dieser wurde aus An und Ae umkristallisiert. 63·1 mg rohes XXIII vom Schmp. 228–235°. Der Mutterlaugenrückstand (195 mg) wurde an 4 g Al_2O_3 chromatographiert. Die mit Pe–Bzl 2:1 eluierten Fraktionen (Nr. 8–12) ergaben aus Ae und Pn 9·8 mg rohes XXII, das nach mehrmaligem Umkristallisieren bei 143–147° schmolz. Die mit Bzl–Chf 9:1, 8:2 und 1:1 eluierten Fraktionen

* Über die stufenweise verlaufende $LiAlH_4$-Reduktion der angularen Cyangruppe ist eine noch nähere Diskussion in der im Referat (1) angegebenen Mitteilung beschrieben.

[6] T. Reichstein und C. W. Shoppee, *Disc. Faraday Soc.* Nr. 7, 305 (1949).

ergaben aus An und Ae weitere 30·1 mg von XXIII vom Schmp. 231–234°. Zur Analyse wurde XXIII weiter umkristallisiert und schmolz danach bei 233–236°. XXIII zeigte eine deutliche positive Zimmermann-Farbreaktion.

dl-D-*Homo*-18-*oxo*-*androstan*-3β-*ol*-17-*on*-3-*acetat*-17-*diäthylketal* XXII. Schmp. 143–147°, I.R ν_{max}^{Nujol} cm^{-1} 2710 (CHO), 1732 (AcO), 1718 (Schulter, CHO), 1248, 1022 (AcO), 1128, 1051 $\left(\begin{matrix}\text{OEt}\\ \text{OEt}\end{matrix}\right)$, (Gef.: C, 71·55; H, 9·74. $C_{26}H_{42}O_5$ (M.G. 434·60) Ber.: C, 71·85; H, 9·74%).

dl-D-*Homo*-18-*aminomethyl*-*androstan*-3β-*ol*-17-*on*-*diacetat* XXIII. Prismen aus An und Ae, Schmp. 233–236°, I.R. ν_{max}^{Nujol} cm^{-1} 3338 (NH), 1734 (AcO), 1715 (C=O), 1685, 1534 (NH—CO), 1237, 1020 (OAc), (Gef.: C, 69·96; H, 9·29; N, 3·02. $C_{24}H_{37}O_4N.1/2H_2O$ (M.G. 412·55) Ber.: C, 69·87; H, 9·28; N, 3·39%).

(2) Umsetzung von XXIII mit abs. Alk und abs. Bzl in Gegenwart von TsOH. 50 mg XXIII wurden mit 12·5 cc abs. Alk und 21 cc abs. Bzl in Gegenwart von 100 mg $TsOH.H_2O$ langsam destilliert, wobei durch Zutropfen eines Mischlösungsmittels von abs. Alk und abs. Bzl im gleichen Verhältnis das ursprüngliche Volumen der Lösung immer konstant behalten wurde. Nach 15 Std. wurde die Lösung abgekühlt, mit 43 mg wasserfreiem NaOAc versetzt und dann im Vakuum eingedampft. Der Rückstand wurde nach Zugabe von etwas Wasser mit Chf extrahiert. Die Chf-Auszüge ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vakuum 54·3 mg Rückstand, der sofort mit 0·6 cc Ac_2O und 1 cc Py acetyliert wurde. Die übliche Aufarbeitung ergab 73·9 mg rohes Acetat, das an 2 g Al_2O_3 (Woelm, Akt. II) chromatographiert wurde. Die mit Pe–Bzl 1:1 und 1:2 eluierten Fraktionen ergaben aus Ae und Pn 16 mg rohes XXIV vom Schmp. 174–175°, das nach weiterem Umkristallisieren bei 179–180° schmolz.

dl-D-*Homo*-17α-*äthoxy*-(17β → 18)-*cycloimin*-*diacetat* XXIV. Schmp. 179–180°, I.R. ν_{max}^{KBr} cm^{-1} 1731 (OAc), 1650 (—CO—N—), (Gef.: C, 70·57; H, 9·67; N, 2·96. $C_{28}H_{41}O_4N.1/2H_2O$ (M.G. 440·6) Ber.: C, 70·87; H, 9·61; N, 3·17%).

II. *Reduktion von* 13β-*Cyan*-17-*äthylenketal*

(1) Reduktion mit 5 Mol $LiAl(OEt)_3H$,[2] $LiAl(OEt)_3H_2$[3] und 3 Mol von $LiAlH_4$. Die Reduktion von dl-18-Nor-D-Homo-13β-cyan-androstan-3β-ol-17-on-3-acetat-17-äthylenketal (IV) mit den oben im Titel genannten Reduktionsmitteln war erfolglos. Die I.R.-Spektren der rohen Produkte zeigten, dass bei Verwendung von 5 Mol $LiAl(OEt)_3H$ nur die 3β-Acetoxylgruppe und bei Verwendung von 5 Mol $LiAl(OEt)_3H_2$ sowie von 3 Mol $LiAlH_4$ ausser der 3β-Acetoxylgruppe die 13β-Cyangruppe nur zu einem sehr geringen Teil angegriffen wurde. Daher wurde auf eine weitere Aufarbeitung verzichtet.

(2) Reduktion mit 10 Mol $LiAl(OEt)_3H_2$. Eine $LiAl(OEt)_3H_2$[3]-Tetrahydrofuranlösung wurde aus 15·5 cc einer 0·312M[7] $LiAlH_4$-Tetrahydrofuranlösung und 350 mg (3·98 m. Mol) Äthylacetat hergestellt. Zu dieser Lösung wurde unter Eiskühlung und Rühren eine Lösung von 160 mg (0·398 m. Mol) IV in 8 cc THF innerhalb von 5 Min. zugetropft und bei der gleichen Temperatur 30 Min., dann bei Raumtemperatur 3·5 Std. gerührt. Das Reaktionsgemisch wurde dann wieder abgekühlt und mit einer Mischlösung von 4 cc einer 0·886M Rochelle'schen Salz-Lösung und 0·36 cc einer 0·5M Weinsäure-Lösung versetzt und dann dreimal mit Chf ausgeschüttelt. Die Chf-Auszüge ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vakuum 214 mg Eindampfrückstand (rohes XIV), dessen I. R.-Spektrum (Nujol) Banden für OH bei 3508, 3458 cm^{-1}, für CH=NH bei 3258, 1635 cm^{-1}, für CN (schwach) bei 2218 cm^{-1} und für Aldehyd (schwach) bei 2700, 1716 cm^{-1} zeigte. Dieses wurde dann in 9 cc Me gelöst, mit 1 cc 2N NaOH versetzt und 5 Min. in einer N_2-Atmosphäre unter Rückfluss gekocht. Die Reaktionslösung wurde mit Essigsäure neutralisiert, im Vakuum eingeengt und dreimal mit Chf extrahiert. Die Chf-Extrakte ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4, Eindampfen im Vakuum 213 mg Eindampfrückstand, der ohne weiteres mit 3 cc Ac_2O und 5 cc trocknem Py bei Raumtemperatur über Nacht acetyliert wurde.

Die übliche Aufarbeitung ergab 224 mg rohes Acetat, das an 8 g Al_2O_3 (Woelm, Akt. III, neutral) chromatographiert wurde. Die mit Pe–Bzl (4:1)–(7:3) eluierten Fraktionen (Nr. 13–20) ergaben aus An und Ae insgesamt 34·8 mg rohen Aldehyd (XV) vom Schmp. 157–172°, der nach weiterem Umkristallisieren bei 170–176° schmolz. Aus den mit Pe–Bzl (3:2)–(3:7) eluierten Fraktionen wurde aus An und Ae 62 mg Ausgangsmaterial vom Schmp. 230–237° erhalten.

[7] Die Bestimmung der Molarität der $LiAlH_4$-Lösung wurde nach der Angabe von John A. Krynitsky, *Analyt. Chem.* **20**, 311 (1948) durchgeführt.

(3) Reduktion mit 10 Mol von $LiAlH_4$. Zu 20 cc einer 0·172M[7] $LiAlH_4$–THF–Lösung wurde unter Eiskühlung sowie kräftigem Rühren eine Lösung von 138·5 mg (0·343 m. Mol) IV innerhalb von 30 Min. getropft und dann bei Raumtemperatur 3 Std. lang gerührt. Die Reaktionslösung wurde dann abgekühlt und mit 10 cc Wasser versetzt. Das Reaktionsgut wurde dann 8 Min. unter Rückfluss gekocht, um das gebildete Imid zu verseifen. Im Vakuum wurde das Lösungsmittel bis ca. zur Hälfte abdestilliert, unter Eiskühlung mit Essigsäure neutralisiert und anschliessend dreimal mit Chf ausgeschüttelt. Die Chf-Auszüge ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vakuum 142·4 mg kristallinisches Produkt, dessen I.R.-Spektrum (Nujol) Banden für OH bei 3580–3520 cm^{-1}, für Aldehyd bei 2730, 2702, 1729 und 1708 cm^{-1} (C—H und C=O, gespalten) und für Ketal bei 1099 cm^{-1} zeigte. Dieses Produkt wurde ohne weiteres mit 1·5 cc Ac_2O und 2·5 cc Py bei Raumtemperatur über Nacht acetyliert. Die übliche Aufarbeitung ergab 160 mg kristallinisches Acetat, das aus An und Ae (abs.) umkristallisiert wurde. Der so erhaltene Aldehyd XV (62·4 mg der ersten Kristalle) schmolz bei 147–173°. Weitere Umkristallisation erhöhte zwar den Schmp. bis auf 151–180°, ergab aber keine Verschärfung. Das ganze Produkt wurde daher einer Chromatographie an 8 g Al_2O_3 (Woelm, Akt. III, neutral) unterworfen. Die mit Pe–Bzl (9:1)–(2:1) eluierten Fraktionen (Nr. 7–28) ergaben aus wenig An und abs. Ae (Peroxydfrei) 50·3 mg Aldehyd (XV) in Platten vom Schmp. 170–179°, aus der Mutterlauge weitere 20·4 mg vom Schmp. 161–173°, das nach weiterem Umkristallisieren bei 172–179° unter Schäumen schmolz. Die mit Bzl eluierten Fraktionen ergaben aus An und Ae ein wenig eines unidentifizierten Produktes vom Schmp. 153–155°, das aber nicht weiter untersucht wurde.

dl-D-*Homo*-18-*oxo-androstan*-3*β*-*ol*-17-*on*-3-*acetat*-17-*äthylenketal* XV. Schmp. 172–179° unter Schäumen, I.R. ν_{max}^{Nujol} cm^{-1} 2742 (mittel, scharf; Aldehyd), 2680 (schwach, scharf; Aldehyd), 1726 (Acetoxy und Aldehyd), 1708 (Aldehyd) 1122, 1099 (Ketal), 1247, 1022 (Acetoxy), U.V. λ_{max}^{EtOH} mμ (ε): 290–292(35), (Gef.: C, 71·00; H, 8·92. $C_{24}H_{36}O_5$ (M.G. 404·53) Ber.: C, 71·25; H, 8·91%).

III. *Huang Minlon'sche Reduktion von* dl-D-*Homo*-18-*oxo-androstan*-3*β*-*ol*-17-*on*-3-*acetat*-17-*äthylen-ketal* (XV)

Eine Mischung von 60 mg XV, 106 mg KOH, 0·3 cc 80% Hydrazinhydrat und 2 cc Triäthylenglykol wurde auf 130–135° erhitzt und 1 Std. bei gleicher Temperatur belassen. Dann liess man die Innentemperatur allmählich bis auf 210–220° ansteigen (30 Min.), während sich das Wasser und das überschüssige Hydrazinhydrat langsam abdestillieren liess; anschliessend wurde die Reaktionslösung 3 Std. auf die gleiche Temperatur erhitzt, wobei eine heftige N_2-Entwicklung beobachtet wurde.

Nach dem Erkalten wurde die Mischung in Eis-Wasser gegossen und dreimal mit Chf ausgeschüttelt. Die Chf-Auszüge ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4 und Eindampfen in Vakuum 65·9 mg kristallinisches Reduktionsprodukt, das aus Alk 25·4 mg rohes XVI in Blättern vom Schmp. 218–222° und aus der Mutterlauge weitere 9·4 mg vom Schmp. 213–219° (total 34·8 mg, 67·3% der Theorie) ergab. Weiteres Umkristallisieren aus An–Ae ergab das reine XVI vom Schmp. 221–223°.

25·8 mg davon wurden mit 0·9 cc Ac_2O und 1·5 cc trockenem Py bei Raumtemperatur über Nacht acetyliert. Die übliche Aufarbeitung ergab 28·6 mg rohes Acetat, das aus Ae und Pn 12·3 mg Acetat XVII in Prismen vom Schmp. 164–166°/173–174° (Doppelt-Schmp.) ergab. Die Mutterlauge wurde im Vakuum eingedampft und der Rückstand erneut aus Me und ein wenig Ae umkristallisiert und ergab weitere 6·2 mg von XVII in Prismen vom Schmp. 164°/170–173° (insgesamt 18·5 mg).

dl-D-*Homo-androstan*-3*β*-*ol*-17-*on*-17-*äthylenketal* XVI. Blätter aus Alk oder An und Ae, Schmp. 221–223°, I.R. ν_{max}^{Nujol} cm^{-1} 3602, 3551 (OH), 1102, 1075 ( ), (Gef.: C, 75·71; H, 10·38. $C_{22}H_{36}O_3$ (M.G. 348·51) Ber.: C, 75·81; H, 10·41%).

dl-D-*Homo-androstan*-3*β*-*ol*-17-*on*-17-*äthylenketal*-3-*acetat* XVII. Prismen aus Ae und Pn, Schmp. 164–166°/173–174°, I.R. ν_{max}^{Nujol} cm^{-1} 1731, 1241, 1028, 1020 (AcO), 1102, 1078 ( ), (Gef.: C, 73·76; H, 9·80. $C_{24}H_{38}O_4$ (M.G. 390·54) Ber.: C, 73·80; H, 9·81%).

IV. *Ketalabspaltung des* dl-D-*Homo-androstan*-3*β*-*ol*-17-*on*-3*β*-*acetat*-17-*ketals* (XVII)

7 mg rohes XVII wurden in 0·5 cc Eisessig gelöst, in der Wärme mit 0·25 cc Wasser versetzt und dann 30 Min. auf 100° erhitzt. Das Lösungsmittel wurde im Vakuum vollständig abdestilliert und der Rückstand (7 mg) wurde dann in Ae aufgenommen, filtriert und im Vakuum eingedampft. Der

Eindampfrückstand wurde erneut aus Ae und Pn umkristallisiert. Dies ergab 6 mg rohes XVIII in Platten (Busch) vom Schmp. 145–148°, welches bei der Mischprobe und dem Vergleich der I.R.-Spektren mit dem im anderen Versuch (Durchlauf-Verfahren s. unten) bereiteten Präparat (Schmp. 154–157°/163°) identisch war.

dl-D-*Homo-androstan-3β-ol-17-on-3-acetat* XVIII. Platten aus An und Ae Schmp. 154–157°/160–163°, I.R. ν_{max}^{Nujol} cm^{-1} 1739 (AcO), 1711 (C=O), 1248, 1239, 1028, (Gef.: C, 76·30; H, 9·77. $C_{22}H_{34}O_3$ (M.G. 346·49) Ber.: C, 76·26; H, 9·89%).

V. *Durchlauf-Verfahren für die Darstellung von* XVIII *ausgehend von* IV

Zu einer Lösung von 1·23 g $LiAlH_4$ in 100 cc abs. THF wurde unter Eiskühlung und Rühren eine Lösung von 1·3 g IV in 30 cc abs. THF innerhalb von 30 Min. eingetropft und dann 3 Std. bei Raumtemperatur gerührt. Unter Eiskühlung wurde das Reaktionsgemisch mit einer Mischlösung von 20 cc einer 0·886M Rochelle'schen Salz-Lösung und 5 cc einer 0·5M Weinsäure-Lösung, dann mit etwas Wasser versetzt und dreimal mit Chf ausgeschüttelt. Die Chf-Auszüge ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vakuum 1·32 g kristallinisches Produkt, dessen I.R.-Spektrum (Nujol) Banden für Hydroxyl bei 3380, 1038 cm^{-1}, für Imid bei 3270 (scharf), 1633 cm^{-1} (stark, scharf) und für Ketal bei 1126, 1098 cm^{-1} zeigte. Das Imid wurde nicht rein isoliert.

Das ganze Produkt wurde in 100 cc Me gelöst, mit 11 cc 2N NaOH versetzt und dann im N_2-Strom 5 Min. unter Rückfluss gekocht. Das Me wurde im Vakuum eingedampft, der Rückstand mit etwas Wasser versetzt und dann dreimal mit Chf ausgeschüttelt. Die Chf-Auszüge ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vakuum 1·223 g kristallinischen Eindampfrückstand (rohen Aldehyd XV, OH anstatt OAc).

Eine Mischung des oben erhaltenen rohen Aldehyds mit 6·1 cc 80-proz. Hydrazinhydrat, 2·14 g KOH und 41 cc Triäthylenglykol wurde langsam erhitzt und eine Stunde lang bei 130–150° gehalten; innerhalb von 30 Min. wurde die Innentemperatur allmählich bis auf 210–220° erhöht und 3 Std. lang bei gleicher Temperatur gehalten. Die Reaktionslösung wurde dann in die etwa zweifache Menge Eiswasser gegossen und dann viermal mit Chf extrahiert. Die Chf-Extrakte wurden zweimal mit Wasser gewaschen und über Na_2SO_4 getrocknet. Eindampfen im Vakuum hinterliess 1·218 g kristallinisches Reduktionsprodukt (XVI), das ohne weiteres einer Ketalabspaltung unterworfen wurde. Es wurde dazu in 40 cc Eisessig gelöst. Unter Schwenken wurden bei 95–100° 20 cc Wasser rasch zugegeben. Die Reaktionslösung wurde dann eine viertel Std. lang bei gleicher Temperatur erhitzt, im Vakuum bis zu einem Drittel des Volumens eingeengt und dann fünfmal mit Chf ausgeschüttelt. Die Chf-Extrakte wurden im Gegenstrom mit 2N Na_2CO_3, dann mit Wasser gewaschen, über Na_2SO_4 getrocknet und im Vakuum eingedampft.

Zuletzt wurde das so erhaltene kristallinische 3-Hydroxy-17-keton (1·006 g) mit 7 cc Ac_2O und 10 cc Py bei Raumtemperatur über Nacht acetyliert. Die übliche Aufarbeitung ergab 1·167 g kristallinisches Rohacetat, das direkt aus An und Ae umkristallisiert wurde. 499 mg erste Kristalle von XVIII in Platten vom Schmp. 152–154° und aus der Mutterlauge weitere 186 mg XVIII vom Schmp 149–153°. Die ersten Kristalle wurden nochmals aus An und Ae umkristallisiert und ergaben das reine XVIII vom Schmp. 154–157°/160–163° (Doppel-Schmp.). Die letzte Mutterlauge wurde im Vakuum eingedampft und der Rückstand (462 mg) wurde an 15 g Al_2O_3 (Woelm, Akt. II, neutral) chromatographiert. Die mit Pe–Bzl (4:1)–(1:1) eluierten Fraktionen ergaben aus Ae und Pn 97·6 mg XVIII vom Schmp. 152–154°/–160° und aus der Mutterlauge weitere 37·4 mg vom Schmp. 150–154°/158–160°. Die mit Pe–Bzl (1:2)–Bzl eluierten Fraktionen ergaben aus Ae und Pn 14·2 mg dl-D-Homo-18-nor-$\Delta^{13(17a)}$-androsten-3β-ol-17-on-3-acetat (I-Acetat) vom Schmp. 134–136° (Mischprobe).

Insgesamt wurden 820 mg dl-D-Homoandrostan-3β-ol-17-on-3-acetat XVIII (73·2% der Theorie bezogen auf IV) erhalten.

VI. *Oxydation von* dl-D-*Homo-18-oxo-androstan-3β-ol-17-on-17-äthylenketal* (XV)

80 mg XV wurden in 15 cc über $KMnO_4$ frisch destillierten An gelöst und dazu wurde unter Rühren bei Raumtemperatur 0·5 cc CrO_3–H_2SO_4 Reagenz (8N)[6] rasch getropft und weiter 20 Min. lang gerührt. Nach Zugabe von fünf Tropfen Me und danach 50 cc Wasser wurde dreimal mit Chf ausgeschüttelt. Die Chf-Auszüge ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vakuum 119 mg Eindampfrückstand, der sofort in 10 cc An gelöst, mit 2 cc 2N HCl versetzt und 30 Min. unter Rückfluss gekocht wurde. Nach Zugabe von 300 mg wasserfreiem

NaOAc wurde die Reaktionslösung im Vakuum eingeengt, mit etwas Wasser versetzt und dann dreimal mit Chf extrahiert. Die Chf-Auszüge ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4, Eindampfen im Vakuum 124·3 mg Eindampfrückstand, der mit 1 cc 2N Na_2CO_3 in 10 cc Me durch Kochen (1·5 Std.) verseift wurde. Das Me wurde im Vakuum abdestilliert, mit etwas Wasser versetzt, und dann dreimal mit Chf ausgeschüttelt. Die Chf-Lösungen wurden zweimal mit 2N NaOH extrahiert und dann mit Wasser gewaschen. Die alkalischen Lösungen und das Waschwasser wurden vereinigt und unter Eiskühlung mit konz. HCl bis auf pH 3 angesäuert. Die ausgeschiedene Säure XIX wurde in Chf aufgenommen. Eindampfen der gewaschenen, dann getrockneten Chf- Extrakte im Vakuum hinterliess 48·5 mg kristallinische Säure XIX, die durch Waschen mit Ae grob gereinigt wurde (37 mg). Andererseits wurde aus den ersten Chf-Auszügen 57·8 mg neutraler Anteil erhalten, der aber nicht weiter untersucht und verworfen wurde. Die so erhaltene rohe Säure (37 mg) wurde mit ätherischer CH_2N_2-Lösung in Dioxan methyliert. Die übliche Aufarbeitung ergab 36·8 mg rohen Ester (XIII), der aus Chf–Ae 12·0 mg XX in Säulen vom Schmp. 186–191°, aus der Mutterlauge weitere 14·6 mg von XX vom Schmp. 184–187° ergab. Der so erhaltene Ester (XX) erwies sich nach einer Mischprobe und dem Vergleich der I.R.-Spektren als identisch mit dem auf anderem Wege hergestellten Präparat (Schmp. 192–198°).[4]

Die zweiten Kristalle (14·6 mg) wurden in 0·5 cc Py gelöst, mit 12 mg Benzoylchlorid versetzt und unter Feuchtigkeitsausschluss über Nacht bei Raumtemperatur stehen gelassen. Die übliche Aufarbeitung ergab 20·6 mg rohes Benzoat, das aus An und Ae 7·6 mg Benzoat (XXI) in Säulen vom Schmp. 228–232° und aus der Mutterlauge weitere 5·0 mg XXI ergab. Die ersten Kristalle wurden nochmals aus An und Ae umkristallisiert und ergaben ein fast reines Präparat vom Schmp. 231–235°, das mit dem authentischen Präparat (Schmp. 234–235°)[4] verglichen wurde (I.R., Mischprobe) und sich ebenfalls als identisch erwies.

Für die Unterstützung bei dieser Arbeit und die wertvolle Diskussion darüber möchte ich Herrn Doktor K. Takeda, dem Direktor dieses Forschungslaboratoriums sowie Herrn Prof. E. Ochiai herzlichst danken. Ich danke auch Herrn T. Terasawa und Herrn T. Aoki für ihre freundliche Hilfe, Herrn Dr. T. Kubota und Herrn Y. Matsui für die optischen Daten und den Herren vom Mikrolaboratorium für die Analysendaten bestens.

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TOTALSYNTHETISCHE UNTERSUCHUNGEN AN STEROIDEN—X*

TOTALSYNTHESE VON RAC. 5α-PREGN-16-EN-20-ON-3β-OL-3-ACETAT UND RAC. 16-ACETYL-ANDROST-16-EN-3β-OL-3-ACETAT

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Zusammenfassung—rac. D-Homo-androstan-3β-ol-17-on-acetat V liess sich durch Umsetzung mit CH_3MgJ und nachfolgende Acetylierung in 17α-Methylhydrin VII verwandeln, das durch Wasserabspaltung mit $POCl_3$ und Pyridin ein Gemisch von zwei isomeren endocyclischen ungesättigten Produkten (XII) ergab. Vier cis-Diole, die durch OsO_4-Oxydation aus XII entstanden sind, konnten sowohl strukturell als auch räumlich aufeinander bezogen werden. Durch Identität des I.R.-Spektrums stellte es sich heraus, dass eines von diesen vier *cis*-Diolen, XVa ein Racemat des natürlichen, optisch aktiven D-Homo-17β-methylandrostan-3β,17α,17aα-triol-3-monoacetat XVb darstellt. Somit wurde bestätigt, dass der Grundkörper der bisher abgeleiteten Zwischenverbindungen denselben sterischen Aufbau wie die natürlichen Steroide besitzt. Ein Paar Diole, XIII und XVa, und ein anderes Paar Diole XIV und XVI liessen sich mit HJO_4 zu den Ketoaldehyden XIX bzw. XX oxydieren, die durch Ringschluss mit Triäthylamin-Acetat in die Endprodukte, rac. 16-Acetyl-Δ^{16}-androsten-3β-ol-acetat XXIIa bzw. rac. Δ^{16}-5α-Pregnen-3β-ol-20-on-acetat XXIIIa übergeführt wurden. XXIIa und XXIIIa erwiesen sich durch Vergleich der I.R.-Spektren, der U.V.-Spektren und der papierchromatographischen Rf-Werte der verseiften Alkohole als die Racematen der entsprechenden natürlichen Verbindungen XXIIb bzw. XXIIIb.

IN den vorhergehenden Mitteilungen dieser Reihe sind die Synthesen von rac. D-Homoandrostan-17-on-3β-ol-acetat (V)* und rac. D-Homo-18-norandrostan-17-on-3β-ol-13β-carbonsäuremethyl-ester (III)[1,*] beschrieben worden, wobei die erste Substanz durch Huang Minlon'sche Reduktion aus der entsprechenden 13β-Aldehydverbindung (I) und die zweite Substanz sowohl durch Abbau der Brückenverbindung II mit verd. Natronlauge als auch durch direkte Oxydation mit CrO_3 aus I erhalten wurde.

In dieser Mitteilung berichten wir über die D-Homoring-Kontraktion von V, die endlich eine erfolgreiche Synthese der rac. Steroide herbeiführte und damit die Richtigkeit der bisher angenommenen räumlichen Konfigurationen der Grundkörper endgültig bewies.

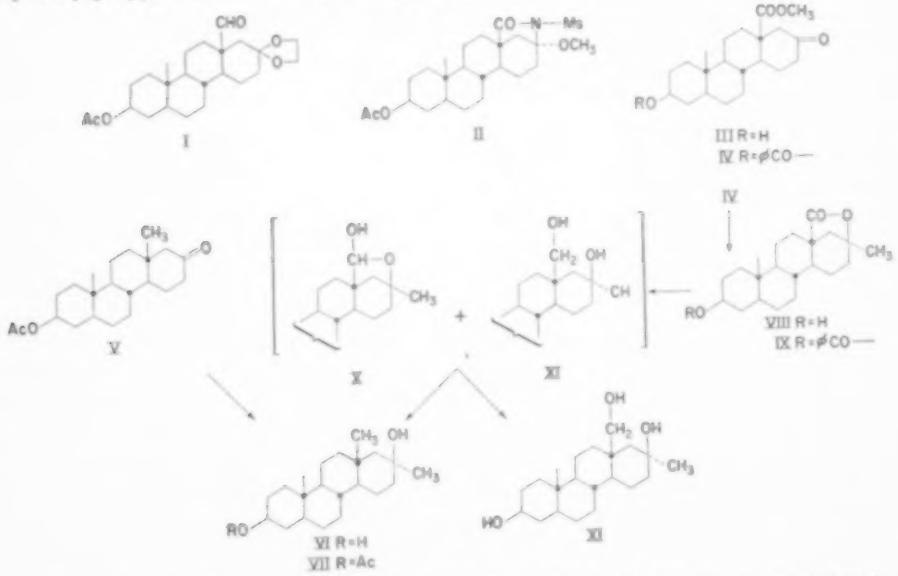
Setzt man V mit einem Überschuss an CH_3MgJ in Benzol um, so bildet sich nach anschliessender Acetylierung ein Methylhydrinacetat VII in guter Ausbeute (82·5%), wobei die Einführung des Methylrestes stereoselektiv von der α-Seite des Steroidmoleküls erfolgt. Dies konnte wie folgt durch Überführung des gleichen VII aus Lakton IX einwandfrei bestätigt werden. III wurde nach der Benzoylierung (IV) mit einem Überschuss an CH_3MgJ in Tetrahydrofuran (THF) umgesetzt, wobei erwartungsgemäss die Benzoylgruppe wegen ihrer relativ geringen Reaktivität der

* IX Mitt.: W. Nagata, *Tetrahedron* **13**, 287 (1961).

[1] W. Nagata, VII. Mitt. dieser Reihe, *Tetrahedron* **13**, 268 (1961).

Carbonyl-Funktion fast nicht, und der 13-Carbonsäureester wegen seiner räumlich gehinderten Lage gar nicht angegriffen wurden. Ferner erzwang die umfangreiche 13-Carbomethoxygruppe die Einführung des Methylrestes an die 17-Carbonylgruppe nur von der α-Seite des Moleküls und somit ergab sich ein stereoselektiver Verlauf der Bildung von γ-Lakton-Benzoat IX, das durch alkalische Verseifung glatt freien Alkohol VIII ergab. Eine bessere Ausbeute von IX wurde aber durch anschliessende Zurückbenzoylierung erreicht. Ausser dem Hauptprodukt IX wurde dabei auch ein hochschmelzendes Produkt (Schmp. über 300°) in kleiner Menge erhalten, das nach seinem I.R.-Spektrum ebenfalls neben der Benzoatgruppe noch eine γ-Laktongruppierung besitzt.* Es wurde demnach vermutet, dass es sich bei ihm um ein dimeres Produkt handelt. Dieses wurde aber nicht weiter untersucht.

In der Absicht, dieses Lakton glatt in ein 18-Oxohemiacetal X zu verwandeln, wurden nun VIII sowie IX in Anlehnung an die Literatur[2] mit der kontrollierten Menge an $LiAlH_4$ in THF reduziert. Es ergab sich nach mehrmalign Versuchen unter verschiedenen Bedingungen, dass die Reduktion hierbei nicht in einheitlicher Weise verlief und immer ein Gemisch von 18-Oxohemiacetal X und 18-Hydroxyverbindung XI lieferte. Dieses Gemisch wurde danach einer Huang Minlon'schen Reduktion unterworfen und nach der chromatographischen Trennung des Reduktionsproduktes an Al_2O_3 wurden zwei Produkte, Diol VI und Triol XI, erhalten. Das erstere ergab nun nach Acetylierung mit Acetanhydrid und Pyridin Monoacetat VII, das sich durch den Misch-schmelzpunkt sowie den Vergleich der I.R.-Spektren mit dem oben erwähnten, aus V abgeleiteten Diolmonoacetat als identisch erwies. Da sich dieses Methylhydrin von γ-Laktonbenzoat IX ableiten liess, besitzt die am C_{17} stehende hydroxylgruppe zweifellos die β-Konfiguration, d.h. die axial-Konformation.

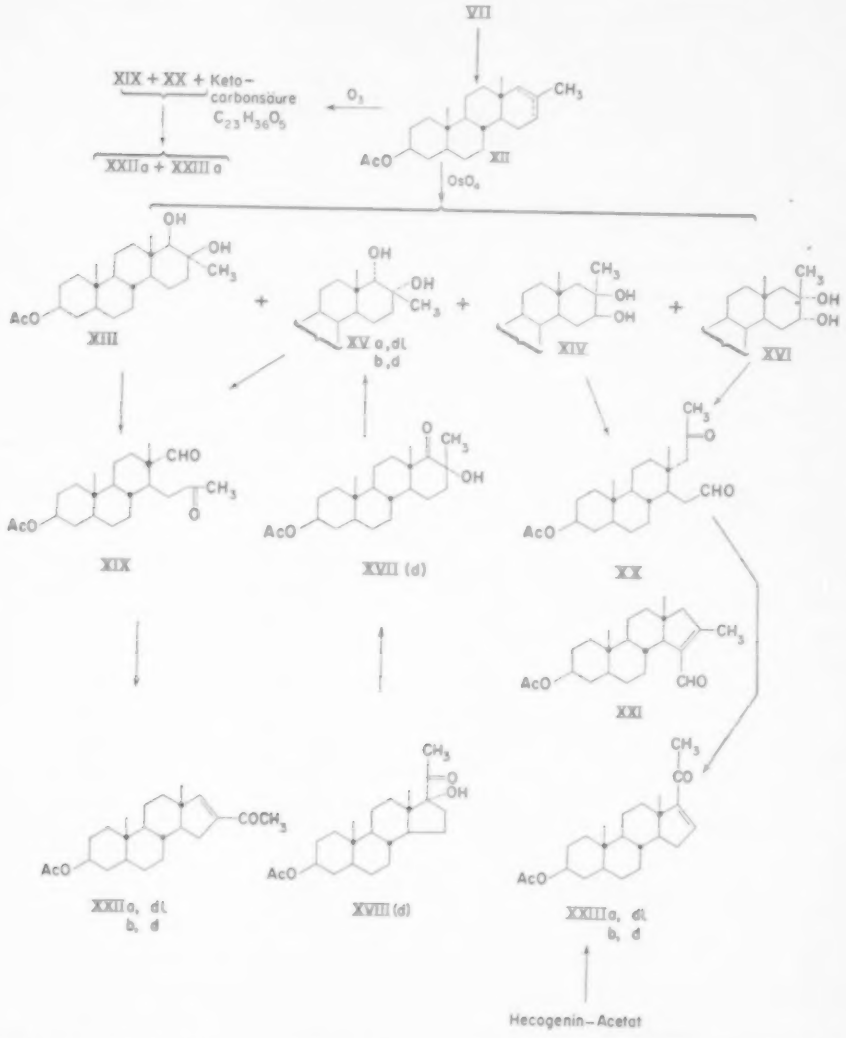


Trans-diaxiale Wasserabspaltung von Methylhydrin VII mit $POCl_3$ und Pyridin

* s. experimenteller Teil.

[2] J. Schmidlin, G. Anner, J.-R. Billeter, K. Heusler, H. Überwasser, P. Wieland und A. Wettstein, *Helv. Chim. Acta* **40**, 2291 (1957).

verlief nicht in gleicher Weise wie beim 3β-Methyl-cholestan-3α-ol,[3] wobei fast ausschliesslich Δ^2-Steroid entstand,[4] und ergab ein Gemisch von Δ^{16}- sowie $\Delta^{17(17a)}$-17-Methyl-D-homo-androstan-3β-ol-acetat (XII). Aus dem Ergebnis der unten erwähnten Oxydation mit OsO_4 zeigte sich, dass das Mischungsverhältnis der beiden ungesättigten Produkte ungefähr 1:1 war und sich dabei fast kein exocyclisch ungesättigtes



Produkt bildete. Die Trennung dieses Gemisches in zwei Komponente durch Al_2O_3-Chromatographie war erfolglos. Daher wurde dieses einer OsO_4-Oxydation unterworfen. Durch die Al_2O_3-chromatographische Trennung des Oydationsproduktes wurden nun vier *cis*-Diole, XIII, XIV, XVa und XVI nach der Reihe der Eluierung

[3] D. H. R. Barton, A. da. S. Campos-Neves und R. C. Cookson, *J. Chem. Soc.* 3500 (1956).
[4] vgl. R. B. Turner, W. R. Meador und R. E. Winkler, *J. Amer. Chem. Soc.* **79**, 4122 (1957).

und in vorwiegender Menge an XVa und XVI erhalten. Durch Identität des I.R.-Spektrums in $CHCl_3$ stellte sich heraus, dass eines von diesen vier *cis*-Diolen, XVa, ein Racemat des optisch aktiven cis-Diols XVb darstellt, das aus dem schon bekannten natürlichen D-Homoannulationsprodukt XVII[5,6] durch Reduktion mit Aluminium-isopropoxyd[7] erhalten wurde. XVII wurde hierbei in Anlehnung an die Angabe von Elphimoff-Felkin *et al.*[5] aus 5α-Pregnan-3β,17α-diol-20-on-acetat XVIII durch Einwirkung von BF_3 hergestellt. Dadurch konnte die Identität der bisher angenommenen räumlichen Anordnung der asymmetrischen Kohlenstoffatome des Grundkörpers mit derjenigen des natürlichen Steroides endgültig bewiesen werden. Ausserdem erwies sich dadurch die Konfiguration der zwei Hydroxylgruppen von XVa als 17α und 17aα. Da sich XIII durch Perjodsäure-Oxydation in ein und denselben aus XVa abgeleiteten Ketoaldehyd XIX verwandelte, orientierte sich die Hydroxylgruppe von XIII nach 17β und 17aβ. Die übrigen zwei Produkte, d.h. XIV und XVI, stellen also die 16,17-Diole dar, weil sich dieses Paar durch HJO_4-Oxydation in einem anderen Ketoaldehyd XX überführen liess. Gemäss des Prinzips des α-Seiten-Angriffs, das hier in der Beziehung zwischen einem anderen Diol-Paar, XIII und XVa schon verwirklicht wurde, dürfte man dem in grosser Menge gebildeten Diol XVI die 16α, 17α, und dem anderen XIV die 16β,17β-Konfiguration zukommen lassen. Somit konnten die vier Diole strukturell sowie räumlich klar aufeinander bezogen werden. Da hierbei kein 17-Hydroxymethyl-17-ol erfasst wurde und da die gesamten Ausbeuten an 16,17- und 17,17a-Diolen ungefähr gleich waren, ist zu schliessen, dass die ursprüngliche ungesättigte Verbindung XII hauptsächlich aus der äquivalenten Menge von Δ^{16}- und $\Delta^{17(17a)}$-17-Methyl-D-homo-androsten-3β-ol-acetat besteht.

Der Ringschluss der schön kristallisierten Ketoaldehyde, XIX und XX zum entsprechenden fünf-gliedrigen D-Ring gelang glatt erst in Anlehnung an die Angabe von W. J. van der Burg *et al.*[8] durch Kochen der Xylol-Lösung in Gegenwart von Triäthylamin-Acetat in einem evakuierten Gefäss und lieferte die gewünschten Endprodukte, nämlich, dl-16-Acetyl-Δ^{16}-androsten-3β-ol-acetat vom Schmp. 163–165° XXIIa bzw. dl-Δ^{16}-5α-Pregnen-3β-ol-20-on-acetat vom Schmp. 167–169° XXIIIa. Dabei erwies sich die Verwendung von Piperidin-Acetat, Natriumäthylat und verd. Natronlauge als Kondensationsmittel in Stickstoff-Atmosphäre bei gewöhnlichem Druck als erfolglos. Ferner wurde gezeigt, dass beim Ringschluss von XX, das isomere Produkt XXI nicht gefasst werden konnte. Dies ist, wie schon R. B. Woodward *et al.*[9] erwähnt haben, auf die durch die räumliche Abstossung zwischen der Aldehyd- und der C_7-Methylengruppe bedingte Unstabilität der Verbindung XXI zurückzuführen.†

Das I.R.-Spektrum von XXIIa in CS_2 war mit demjenigen des aus dem Diol XVb nach den gleichen Reaktionsfolgen erhaltenen, optisch aktiven 16-Acetyl-Δ^{16}-androsten-3β-ol-acetates XXIIb, wie aus der Abbildung 2 zu ersehen ist, in allen Einzel-

† In dem bicyclischen Ringsystem ist keine solche Wechselwirkung vorhanden und daher bildet sich das Produkt von Typs XXI. vgl. N. L. Wendler und H. L. Slates, *J. Amer. Chem. Soc.* **80**, 3937 (1958).

[5] I. Elphimoff-Felkin und A. Skrobak, *Bull. Soc. Chim. Fr.* 742 (1959).
[6] D. K. Fukushima, S. Dobriner, M. S. Heffler, T. H. Kritchevsky, F. Herling und G. Roberts, *J. Amer. Chem. Soc.* **77**, 6585 (1955).
[7] vgl. N. L. Wendler und D. Taub, *J. Org. Chem.* **23**, 953 (1958).
[8] W. J. van der Burg, D. A. van Dorp, O. Schindler, C. M. Siegmann und S. A. Szpilfogel, *Rec. Trav. Chim.* **77**, 171 (1958).
[9] R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler und W. M. McLamore, *J. Amer. Chem. Soc.* **74**, 4223 (1952).

heiten identisch. Die U.V.-Spektren der beiden waren ebenfalls identisch. Die weitere Identifizierung wurde dann durch Papierchromatographie der beiden verseiften, freien Alkohole durchgeführt, wobei beide Substanzen in einem System Formamid-Aceton (1:2)/Toluol-Cyclohexan (1:3) denselben *Rf*-Wert 0·85 ergaben.

Ferner lieferte das andere synthetisierte rac. Steroid, dl-Δ^{16}-5α-Pregnen-3β-ol-20-on-acetat XXIIIa ebenfalls ein vollständig identisches I.R.- (Abb. 1) sowie U.V.-Spektrum wie dasjenige des in der Literatur schon bekannten, optisch aktiven Δ^{16}-5α-Pregnen-3β-ol-20-on-acetates XXIIIb, das nach der Angabe von A. F. B. Cameron *et al.*[10] aus Hecogenin-Acetat hergestellt wurde. Die Beziehung beider Substanzen wurde auch durch denselben *Rf*-Wert 0·87 ihrer verseiften freien Alkohole auf einem Papier-chromatogramm in dem oben beschriebenen Lösungsmittelsystem völlig gesichert.

Ausserdem konnten XXIIa und XXIIIa aus XII durch Ozonisierung und nachfolgenden Ringschluss der gebildeten rohen Ketoaldehyde, wie oben, mit Triäthylamin-Acetat in Xylol-Lösung, wenn auch in etwas schlechterer Ausbeute, gleicherweise erhalten werden, wobei sich in der ersten Oxydationsstufe bei der Verwendung von einem Überschuss an O_3 eine Ketocarbonsäure vom Schmp. 226–230° in einer Ausbeute von 15–20% bildete. Diese stellte scheinbar ein weiteres Oxydationsprodukt von Ketoaldehyd XIX oder XX dar und die Analysenwerte stimmten auch damit sehr gut überein. Dieses Produkt wurde aber nicht weiter untersucht.

BESCHREIBUNG DER VERSUCHE

Alle Schmp. sind auf dem Kofler-Block "Monoskop" (Fa. Hans Bock, Frankfurt/Main, Deutschland) bestimmt worden und sind korrigiert. Die Sdp. sind unkorrigiert. Wenn nicht anders angegeben, wurden Substanzproben zur Elementaranalyse 3 Std. lang bei 1 Torr über P_2O_5 bei Zimmertemperatur bis zu 60°, 70–90° und 100–120° je nach dem Schmp. bis zu 120°, 180° und mehr getrocknet. Die Adsorptionschromatogramme wurden nach der Durchlaufmethode[11] durchgeführt. Es werden folgende Abkürzungen benützt; Pe = Petroläther, Ae = Äther, Alk = Alkohol, An = Aceton, Bzl = Benzol, Chf = Chloroform, Me = Methanol, Pn = Pentan, Py = Pyridin, Akt. = Aktivität.

I. D-*Homosteroid-Synthese aus* 13β-*Carbonsäureester*

(1) *Benzoylierung von* D-*Homo*-18-*nor*-13β-*carbomethoxy-androstan*-3β-*ol*-17-*on* (III)

100 mg III wurden in 0·5 cc trocknem Py gelöst, unter Eiskühlung sowie Schwenkung mit 80·5 mg (2 Äquiv.) Benzoylchlorid versetzt und dann bei Raumtemperatur über Nacht stehen gelassen.

Die übliche Aufarbeitung ergab 140·4 mg eines kristallinischen rohen Benzoats IV, das aus An und Ae umkristallisiert wurde. Erste Kristalle: 99·6 mg vom Schmp. 234–237°, zweite Kristalle aus der Mutterlauge: 14·0 mg vom Schmp. 226–234°: Totalausbeute, 113·6 mg (88% der Theorie).

dl-D-Homo-18-nor-13β-carbomethoxyandrostan-3β-ol-17-on-3-benzoat (IV). Aus An und Ae, Schmp. 234–237°, I.R. ν_{max}^{Nujol} cm^{-1} 3070 (φ), 1725 (COOCH$_3$), 1710 (φ—CO—O—, C=O), 1604, 1586, 1490, 713 (φ), (Gef.: C, 74·67; H, 8·07. $C_{28}H_{36}O_5$ (M.G. 452·57) Ber.: C, 74·30; H, 8·02%).

(2) *Umsetzung von* IV *mit* CH_3MgJ

430 mg IV wurden in 20 cc wasserfreiem Tetrahydrofuran (THF) gelöst. Dazu wurde unter Rühren eine ätherische Grignard-Lösung, hergestellt aus 231 mg Mg und 1·5 g CH_3J in 6 cc abs. Ae, bei Raumtemperatur während 5 Std. langsam eingetropft. Nach weiterem einstündigem Rühren bei Raumtemperatur wurde das Reaktionsgemisch 1 Std. lang unter Rückfluss gekocht.

[10] A. F. B. Cameron, R. M. Evans, J. C. Hamlet, J. S. Hunt, P. G. Jones und A. G. Long, *J. Chem. Soc.* 2807 (1955).

[11] T. Reichstein und C. W. Shoppee, *Disc. Faraday Soc.* Nr. 7, 305 (1949).

Unter Abkühlung mit Eiswasser wurde das Gemisch mit 5 cc Wasser, dann mit 10 cc 2N Salzsäure zersetzt und dann dreimal mit Chf ausgeschüttelt. Die Chf-Auszüge ergaben nach Waschen mit 2N Na_2CO_3, dann mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vak. 507·3 mg Eindampfrückstand, der sofort mit 730 mg Benzoylchlorid und 5 cc Py bei Raumtemperatur über Nacht zurückbenzoyliert wurde. Die übliche Aufarbeitung ergab 871·7 mg. rohes Benzoat, das an 15 g Al_2O_3 (Woelm, Akt. II, neutral) chromatographiert wurde.

Die Fraktionen Nr. 2–9 (eluiert mit Pe–Bzl (1:1)–(1:2)) ergaben aus An und Ae 159·3 mg rohes Lakton-Benzoat (IX) in feinen Nadeln vom Schmp. 244–246°, aus der Mutterlauge weitere 17·9 mg vom Schmp. 239–246°, das nach nochmaligem Umkristallisieren bei 246–248° schmolz. Die Fraktionen Nr. 15–18 (eluiert mit Bzl–Chf (9:1)) ergaben aus An und Ae 28·2 mg hochschmelzende Kristalle (Schmp. über 300°). Es wurde nach dem I.R.-Spektrum sowie den Analysenwerten vermutet, dass es sich bei diesen um ein dimeres Lakton-Benzoat $C_{55}H_{68}O_8$ handelt.

dl-D-Homo-18-nor-17α-methyl-17β-hydroxy-3α-benzoyloxy-androstan-13β-carbonsäure-(13 → 17) β-lakton (IX). Feine Nadeln aus An und Ae, Schmp. 246–248°, I.R. ν_{max}^{Nujol} 3070 (ϕ), 1767 (γ-Lakton), 1717 (ϕ—CO—O—), 1623, 1609, 1589, 1493, 718 (ϕ), 1282, 1124 (ϕ—CO—O—), I.R. ν_{max}^{Chf} cm^{-1} 1763 (γ-Lakton) 1712, 1280, 1122 (ϕ—CO—O—), (Gef.: C, 77·07; H, 8·28. $C_{28}H_{36}O_4$ (M.G. 436·57) Ber.: C, 77·03; H, 8·31%).

Dimeres Lakton Benzoat (WN 227). Feine Kristalle aus An und Ae, Schmp. über 300°, I.R. ν_{max}^{Nujol} 3070 (ϕ), 1767 (γ-Lakton), 1728, 1714 (ϕ—CO—O—), 1607, 1590, 1495, 718 (ϕ), 1285, 1117 (ϕ—CO—O—), I.R. ν_{max}^{Chf} cm^{-1} 1768 (γ-Lakton), 1714, 1280, 1122 (ϕ—CO—O—), Gef.: C, 75·72, 75·57; H, 8·42, 8·50. Kristallwasserbestimmung, Trocknen über P_2O_5 bei 100° 2 Std. dann bei 130° 1 Std. bei Torr. ergab keinen Gewichtverlust. $C_{55}H_{68}O_8.H_2O$ (dimeres Laktonbenzoat) (M.G. 875·11) C, 75·48; H, 8·06. $C_{56}H_{72}O_9$ (M.G. 889·14) C, 75·62; H, 8·17%.

(3) *Verseifung von* IX

41 mg IX wurden in 2·5 cc Dioxan und 2·5 cc Me gelöst, mit einer Lösung von 61 mg KOH in 0·5 cc Wasser versetzt und dann 3 Std. im N_2-Strom unter Rückfluss gekocht. Unter Eiskühlung wurde die Reaktionslösung mit konz. HCl angesäuert und dann dreimal mit Chf extrahiert. Die Chf-Auszüge ergaben nach Waschen mit 2N Na_2CO_3, dann mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vak. 35·8 mg kristallinisches Verseifungsprodukt, das aus An und Ae umkristallisiert wurde.

21·3 mg VIII in Platten vom Schmp. 225–226°, aus Mutterlauge weitere 8·4 mg vom Schmp. 218–220°.

dl-D-Homo-18-nor-17α-methylandrostan-3β,17β-diol-13β-carbonsäure-(13 → 17)β-lakton (VIII). Platten aus An und Ae, Schmp. 225–226°, I.R. ν_{max}^{Nujol} cm^{-1} 3530 (OH), 1746 (γ-Lakton), (Gef.: C, 75·67; H, 9·63. $C_{21}H_{32}O_3$ (M.G. 332·47) Ber.: C, 75·86; H, 9·70%).

(4) *Lithiumaluminiumhydrid- und nachfolgende Huang Minlon'sche Reduktion von* IX

(i) $LiAlH_4$-*Reduktion.* Zu einer Lösung von 148 mg IX in 10 cc wasserfreiem THF wurde unter Eiskühlung sowie Rühren 1·8 cc einer ätherischen 0·248M $LiAlH_4$-Lösung[12] (1·25 Mol Äquiv.) innerhalb von 15 Min. hinzugetropft und dann 3 Std. unter Rückfluss gekocht. Nach Abkühlung mit Eiswasser wurde das Reaktionsigemisch durch Hinzugabe einer Mischung von 7 cc 0·88M Rochelle'scher Salz-Lösung und 5 cc 0·25M Weinsäure-Lösung zersetzt und dann fünfmal mit Chf ausgeschüttelt. Die Chf-Auszüge ergaben nach Waschen mit Wasser (zweimal), Trocknen über Na_2SO_4 und vollständigem Eindampfen im Vak. 134·4 mg Rückstand, dessen I.R.-Spektrum (Nujol) Banden für Hydroxyl bei 3500 cm^{-1} und 3310 cm^{-1}, stark geschwächte Lakton-Bande (ca. ein Zehntel) bei 1768 cm^{-1}, zwei Carbonyl-Banden (für Benzoat?) bei 1715 cm^{-1} und 1694 cm^{-1}, Banden für Benzoat bei 1285, 1122 cm^{-1} und für Bzl bei 1605, 1588, 717 cm^{-1} zeigte, woraus vermutet wurde, dass der γ-Lakton-Ring bevorzugt durch $LiAlH_4$ angegriffen wurde. Dieser rohe Eindampfrückstand wurde sofort einer Huang Minlon-Reduktion unterworfen.

Unter den noch milderen Reduktionsbedingungen (z.B. bei der Verwendung von 0·75- sowie 1-Mol. Äquiv. $LiAlH_4$ und bei Raumtemperatur) wurde das Ausgangsmaterial als Hauptprodukt zurückerhalten.

[12] J. A. Krynitsky, *Analyt. Chem.* **20**, 311 (1948).

Anwendung dieser Reduktion auf Hydroxylakton VIII ergab ein ungefähr gleiches Resultat.

(ii) *Huang Minlon-Reduktion.* 130 mg des oben beschriebenen Eindampfrückstands wurde mit 207 mg KOH und 0·38 cc 80%-Hydrazinhydrat in 2·8 cc Triäthylenglykol langsam erhitzt, wobei die Innentemperatur 30 Min. bei 120–140° gehalten, innerhalb von 1 Std. bis auf 210° erhöht und dann 2·5 Std. bei 210–220° belassen wurde.

Die Reaktionslösung wurde auf Eiswasser gegossen und fünfmal mit Chf extrahiert. Die Chf-Auszüge wurden der Reihe nach mit Wasser, 2N HCl, 2N Na_2CO_3 und Wasser gewaschen und dann über Na_2SO_4 getrocknet. Eindampfen im Vak. hinterliess 106·3 mg neutralen Anteil. Die wässrige Phase wurde unter Eis-Kühlung mit konz. HCl bis auf Kongograu angesäuert und dann mit Chf extrahiert. Die Chf-Auszüge ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vak. 26·5 mg sauren Anteil, der aber nicht weiter verarbeitet wurde.

Der neutrale Anteil (106·3 mg) wurde einer Al_2O_3-Chromatographie (4 g, Woelm Akt. II, neutral) unterworfen. Die Fraktionen Nr. 14–22 (eluiert mit Bzl–Chf (1:2)–Chf) ergaben aus An 6 mg des gewünschten Reduktionsproduktes VI in Nadeln vom Schmp. 224–228°, das ohne weiteres acetyliert wurde (s. unten).

Die Fraktionen Nr. 24–38 (eluiert mit Chf–Me (99:1)–(95:5)) ergaben aus Chf, Me und An insgesamt 50·3 mg Platten oder Prismen vom Schmp. 273–278°, die nach nochmaligem Umkristallisieren keine Schmp.-Erhöhung ergaben und deren I.R.-Spektrum sowie Analysenwerte mit Triol XI gut übereinstimmten. Dieses Triol bildete sich offenbar schon bei der $LiAlH_4$-Reduktion.

Das erste Produkt (VI, 6 mg) wurde mit 0·3 cc Acetanhydrid und 0·5 cc Py bei Raumtemperatur über Nacht acetyliert. Die übliche Aufarbeitung ergab 6·0 mg rohes Acetat (VII), das aus An (wenig) und Ae 5·4 mg Platten vom Schmp. 140–144°/157–158° (beim ersten Schmp. in Form von dicken langen Platten umkristallisierte) ergab. Dieses Produkt (VII) war in jeder Hinsicht (Mischprobe, I.R.-Spektrum in Chf) mit dem auf anderem Wege hergestellten Präparat (s. unten) identisch.

dl-D-Homo-17α-methyl-androstan-3β,17β,18-triol (XI). Platten aus Chf, Me und An, Schmp. 273–278°, I.R. ν_{max}^{nujol} cm^{-1} 3485, 3435, 3315 (OH), 1040 (C—O), (Gef.: C, 74·74; H, 10·68. $C_{21}H_{36}O_3$, (M.G. 336·50) Ber.: C, 74·95; H, 10·78%).

II. *Synthese der Racemate*

(1) *Umsetzung von V mit Methylmagnesiumjodid*

(i) *In Tetrahydrofuran bei Raumtemperatur.* Die Umsetzung von V mit einem Überschuss an CH_3MgJ in THF Lösung bei Raumtemperatur ergab hauptsächlich das Ausgangsmaterial und ein wenig vom Umsetzungsprodukt (VII).

(ii) *In Bzl bei Rückflusstemperatur.* Zu einer ätherischen Methylmagnesiumjodid-Lösung, bereitet aus 356 mg (10·48 m. Mol) Magnesium und 2·07 g (10·48 m. Mol) Methyljodid in 14 cc abs. Ae, wurde unter Rühren und Eiskühlung eine Lösung von 513·7 mg (1·48 m. Mol) dl-D-Homoandrostan-3β-ol-17-on-3-acetat (V) in 15 cc abs. Bzl innerhalb von 20 Min. eingetropft. Das Eis-Bad wurde dann entfernt und das Reaktionsgut weiter 1 Std. lang bei Raumtemperatur gerührt, anschliessend wurde der Ae abdestilliert, mit 30 cc abs. Bzl versetzt und 2·5 Std. lang unter Rückfluss gekocht.

Das Reaktionsgemisch wurde dann im Eis-Bad abgekühlt, durch Zugabe von 20 cc N HCl zersetzt und fünfmal mit einer Mischung von Chf (3 Vol.) und Me (1 Vol.) erschöpfend extrahiert. Die Auszüge ergaben nach Waschen mit halb gesättigter Natriumsulfat-Lösung (zweimal), Trocknen über Na_2SO_4 und Eindampfen im Vak. 623·9 mg Rohprodukt, das ohne weiteres mit 4 cc Ac_2O und 5 cc trocknem Py bei Raumtemperatur über Nacht acetyliert wurde. Die übliche Aufarbeitung ergab 671·6 mg rohes Acetat, das aus An und Ae umkristallisiert wurde und 291·1 mg VII in Prismen vom Schmp. 142–144°/162–163° (Doppelschmp.) und aus der Mutterlauge weitere 101·1 mg VII vom Schmp. 142–144°/157–162° ergab. Es wurde beobachtet, dass das bei 142° halb geschmolzene Produkt (VII) beim weiteren Erhitzen in langen Platten erstarrte und bei 162–163° völlig schmolz. Dieses Produkt erwies sich in einer Mischprobe und dem Vergleich der I.R.-Spektren als identisch mit dem auf anderem Wege hergestellten Präparat (VII, s. oben). Daraus folgte, dass die neu an C_{17} eingeführte Methylgruppe die α-Konfiguration (äquatorial) besass. Der letzte Mutterlaugenrückstand (268·9 mg) wurde an 8 g Al_2O_3 (Woelm Akt. II, neutral) chromatographiert, woraus weitere 50·1 mg VII vom Schmp. 142–144°/163–164·5° (aus den mit Pe–Bzl 4:1–2:1 eluierten Fraktionen, Nr. 6–14) und 9·7 mg Ausgangsmaterial (aus den mit Pe–Bzl 1:1–Bzl eluierten Fraktionen, Nr. 17–23) erhalten wurden. Die Ausbeute von VII betrug dann 442·3 mg (82·5% der Theorie). Zur

Analyse wurde dieses im Vak. über P_2O_5 bei 100–110° getrocknet und schmolz bei 161–163° ohne sich in Kristalle umzuformen.

dl-D-Homo-17α-methyl-androstan-3β,17β-diol-3-acetat (VII). Prismen oder Platten aus An und Ae, Schmp. 161–163° (nach Trocknen), I.R. ν_{max}^{cbf} cm^{-1} 3625, 3505 (OH), 1726, 1249, 1025 (AcO), (Gef.: C, 76·22; H, 10·60. $C_{23}H_{38}O_3$ (M.G. 362·53) Ber.: C, 76·19; H, 10·57%).

(2) *Wasserabspaltung von* VII

540 mg VII wurden in 4 cc trocknem Py gelöst. Unter Eiskühlung und Schwenken wurden 0·5 cc Phosphoroxychlorid dazu eingetropft und dann 40 Min. auf 60–65° erwärmt. Die übliche Aufarbeitung ergab 549·3 mg kristallinisches Rohprodukt, das aus Alk umkristallisiert wurde. 437·6 mg Kristalle vom Schmp. 119–120°. Der Mutterlaugen-rückstand (86·7 mg) wurde an 4 g Al_2O_3 chromatographiert und ergab weitere 13·2 mg Kristalle vom Schmp. 120–122° oder 107–111° je nach der Qualität. Diese Kristalle stellten offenbar ein Gemisch von zwei endocyclischen ungesättigten Isomeren ($\Delta^{16(17)}$- und $\Delta^{17(17a)}$-Verbindungen) dar. Die Trennung durch Chromatographie an Al_2O_3 war erfolglos.

Das aus der Al_2O_3-Säule früher eluierte Kristallisat schmolz bei 132–136° und das später eluierte bei 120–123°. Die Oxydation dieses Gemisches mit OsO_4 und nachfolgende Untersuchung zeigten, dass es sich bei ihm um ein Gemisch von $\Delta^{16(17)}$- und $\Delta^{17(17a)}$-ungesättigten Produkten im Verhältnis von 1:1 handelt und dieses fast kein exocyclisches ungesättigtes Isomeres enthielt (s. unten).

Ein Gemisch von dl-$\Delta^{16(17)}$-und $\Delta^{17(17a)}$-17-methyl-D-homo-androsten-3β-ol-3-acetat (XII), Schmp. 132–136° oder Schmp. 120–123°, gelbe Färbung mit Tetranitromethan, I.R. ν_{max}^{Nujol} cm^{-1} 1729, 1260, 1238, 1043, 1038 (OAc), 831 $\left(>C=C<^{H}\right)$, (Gef.: C, 80·42; H, 10·44. $C_{23}H_{36}O_2$ (M.G. 344·52) Ber.: C, 80·18; H, 10·53%).

(3) OsO_4-*Oxydation von* XII

390 mg (1·132 m. Mol) XII wurden in 13 cc abs. Bzl gelöst, mit 343 mg (1·36 m. Mol) OsO_4, dann mit 0·4 cc Py versetzt und dann bei Raumtemperatur 24 Std. stehen gelassen, wobei braune Niederschläge auftraten.

Nachdem die Niederschläge bei Zugabe von 22 cc Dioxan in die Lösung gingen, wurde das gebildete Osmat durch Einleiten von H_2S-Gas zersetzt. Das ausgefallene Osmiumsulfid wurde durch eine Celit-Schicht abfiltriert und mit Chf mehrmals gespült. Das Filtrat und das Wasch-Chf wurden vereinigt und im Vak. eingedampft. Der Eindampfrückstand wurde erneut in Chf aufgenommen und die Chf-Auszüge wurden mit Wasser gewaschen, über Na_2SO_4 getrocknet und dann im Vak. eingedampft. Der so erhaltene kristallinische Rückstand (422·9 mg) wurde an 30 g Al_2O_3 (Woelm Akt. II) chromatographiert. Die Fraktionen Nr. 10–14 (eluiert mit Bzl–Chf (4:1)) ergaben aus An (wenig), Ae und Pn 13·2 mg XIII in Nadeln oder Säulen vom Schmp. 230–237°, das nach weiterem Umkristallisieren bei 240–242° schmolz.

Die Fraktionen Nr. 16–21 (eluiert mit Bzl–Chf (7:3)) ergaben aus An (wenig), Ae und Pn 39·1 mg XIV in Prismen vom Schmp. 204–206°, das nach weiterem Umkristallisieren bei 205–206° schmolz.

Die Fraktionen Nr. 22–26 (eluiert mit Bzl–Chf (7:3)–(1:1)) ergaben ein Gemisch von XIV und XVa vom Schmp. 177–181°.

Die Fraktionen Nr. 27–38 (eluiert mit Bzl–Chf (1:1)–(3:7)) ergaben aus An (wenig), Ae und Pn 131·1 mg XVa in seidenförmigen Kristallen vom Schmp. 203–204°/206–207° (Doppelschmp.). Es wurde beobachtet, dass dieses Produkt zuerst bei 203–204° schmolz und dann beim Erkalten in Prismen erstarrte, die bei ein wenig höherer Temperatur, nämlich bei 206–207° schmolzen. Dieser Polymorphismus wurde auch dabei beobachtet, wenn die seidenförmige unstabilere Form in An-Lösung gekocht wird. Dabei wandelte sie sich spontan in Prismen, eine stabilere Form um. Die I.R.-Spektren von beiden Formen in Chf waren identisch. Die Fraktionen Nr. 41–46 (eluiert mit Chf–Chf–Me (95:5)) ergaben aus An und Ae 98·1 mg XVI in Säulen vom Schmp. 223–231°, das nach Umkristallisieren bei 227–230° schmolz.

dl-17α-Methyl-D-homo-androstan-3β,17β,17aβ-triol-3-acetat (XIII). Nadeln oder Säulen aus An (wenig), Ae und Pn, Schmp. 240–242°, I.R. ν_{max}^{Chf} cm^{-1} 3600–3300 (OH), 1726, 1250, 1027 (OAc), (Gef.: C, 73·09; H, 10·09. $C_{23}H_{38}O_4$ (M.G. 378·53) Ber.: C, 72·97; H, 10·12%).

dl-17α-Methyl-D-homo-androstan-3β,16β,17β-triol-3-acetat (XIV). Prismen aus An (wenig), Ae und Pn, Schmp. 205–206° I.R. ν_{max}^{Chf} cm^{-1} 3578, 3550–3300 (OH), 1727, 1250, 1025 (OAc), (Gef.: C, 73·22; H, 10·07. $C_{23}H_{38}O_4$ (M.G. 378·53) Ber.: C, 72·97; H, 10·12%).

dl-17β-Methyl-D-homo-androstan-3β,17α,17aα-triol-3-acetat (XVa). Seidenartige Nadeln oder Prismen aus An (wenig), Ae und Pn, Schmp. 203–204° (seidenartige Nadeln), sowie 206–207° (Prismen), I.R. ν_{max}^{Chf} cm^{-1} 3559, 3520–3300 (OH), 1727, 1252, 1027 (OAc), I.R.-Spektren von beiden Formen sowie von dem natürlichen d-Körper (s. unten) in Chf waren in allen Einzelheiten identisch. (Gef.: C, 72·42; H, 9·98. $C_{23}H_{38}O_4$ (M.G. 378·53) Ber.: C, 72·97; H, 10·12%).

dl-17β-Methyl-androstan-3β,16α,17α-triol-3-acetat (XVI). Säulen aus An und Ae, Schmp. 227–230°, I.R. ν_{max}^{Chf} cm^{-1} 3576, 3546–3300 (OH), 1727, 1253, 1026 (OAc), (Gef.: C, 73·27; H, 10·15. $C_{23}H_{38}O_4$ (M.G. 378·53) Ber.: C, 72·97; H, 10·12%).

(4) *Perjodsäure-Oxydation von* XVI *und* XIV

(i) *von* XVI. 92 mg (0·243 m. Mol) XVI wurden in 2 cc Me und 2·5 cc Dioxan gelöst, mit einer Lösung von 77 mg (0·338 m. Mol) $HJO_4.2H_2O$ in 1·5 cc Wasser versetzt und dann bei Raumtemperatur 1 Std. stehen gelassen.

Nach Einengen im Vak. wurde das Reaktionsgemisch in Wasser gegossen und dann dreimal mit Chf ausgeschüttelt. Die Chf-Auszüge wurden mit 2N Na_2CO_3-Lösung und Wasser gewaschen, über Na_2SO_4 getrocknet und dann im Vak. eingedampft.

Eine Portion des rohen kristallinischen Eindampfrückstands (90 mg) wurde zur Identifizierung aus peroxidfreiem Ae und Pn umkristallisiert. Schöne Nadeln (XX) vom Schmp. 112–115°. Der Mutterlaugenrückstand wurde mit den ersten rohen Kristallen vereinigt und zur weiteren Cyclisierung verwendet. Er zeigte mit Tetrazoliumsalzen in alkalischer Lösung nur eine schwache Rot-Färbung.

(ii) *von* XIV. 28·3 mg (0·0748 m. Mol) XIV wurden in 1 cc Dioxan und 1·2 cc Me gelöst, mit einer Lösung von 23·7 mg (0·104 m. Mol) $HJO_4.2H_2O$ in 0·7 cc Wasser versetzt und dann bei Raumtemperatur 1·5 Std. stehen gelassen. Nach gleicher Aufarbeitung wie bei 4-(i) wurde 29·0 mg Eindampfrückstand erhalten, der aus peroxydfreiem Ae und Pn 9·3 mg XX in Nadeln vom Schmp. 112–115° und aus der Mutterlauge weitere 4·9 mg XX vom Schmp. 105–112° ergab. Dieses Produkt erwies sich in einer Mischprobe sowie dem Vergleich der I.R.-Spektren (in Chf) als identisch mit dem bei 4-(i) erhaltenen Präparat.

Ketoaldehyd (XX). Nadeln aus Ae, Schmp. 112–115°, I.R. ν_{max}^{Chf} cm^{-1} 2711 (Mittel, scharf) (—CHO), 1725 (AcO, CHO), 1714 (Schulter, C=O). 1245, 1029 (AcO), (Gef.: C, 73·42; H, 9·62. $C_{23}H_{36}O_4$ (M.G. 376·52) Ber.: C, 73·36; H, 9·64%).

(5) *Perjodsäure-Oxydation von* XVa *und* XIII

(i) *von* XVa. 127 mg (0·336 m. Mol) XVa wurden in 4 cc Me und 2·5 cc Dioxan gelöst, mit einer Lösung von 106 mg (0·465 m. Mol) $HJO_4.2H_2O$ in 1·5 cc Wasser versetzt und dann bei Raumtemperatur 1 Std. stehen gelassen. Die gleiche Aufarbeitung wie bei 4-(i) ergab 125 mg eines kristallinischen rohen Ketoaldehyds (XIX). Eine Portion davon wurde zur Identifizierung aus peroxydfreiem Ae und Pn umkristallisiert. Prismen vom Schmp. 118·5–120° (XIX). Mischschmp. mit dem isomeren Ketoaldehyd vom Schmp. 112–115° (XX) war 86–107°. Der Mutterlaugenrückstand wurde mit den ersten rohen Kristallen vereinigt und weiter verarbeitet (s. unten). Dieses Produkt zeigte bei der Tetrazolium-Probe ebenfalls nur eine schwache Rotfärbung.

(ii) *von* XIII. 14·1 mg (0·037 m. Mol) XIII wurden in 0·5 cc Dioxan und 0·7 cc Me gelöst, mit einer Lösung von 11·8 mg (0·052 m. Mol) $HJO_4.2H_2O$ in 0·7 cc Wasser versetzt und dann bei Raumtemperatur 6 Std. stehen gelassen. Die gleiche Aufarbeitung wie bei 4-(i) ergab 15 mg Eindampfrückstand, der aus peroxydfreiem Ae und Pn fraktioniert umkristallisiert wurde. Zuerst wurden 1·8 mg Ausgangsmaterial vom Schmp. 223–239° oder 230–240° je nach der Qualität erhalten (Mischprobe). Aus der Mutterlauge wurden 6·6 mg XIX vom Schmp. 114–118° erhalten, welches in einer Mischprobe sowie dem Vergleich der I.R.-Spektren (Chf) mit dem oben beschriebenen Ketoaldehyd (XIX) identisch war.

Ketoaldehyd (XIX). Prismen aus peroxydfreiem Ae und Pn, Schmp. 118·5–120°, I.R. ν_{max}^{Chf} cm^{-1} 2697 (CHO), 1722 (OAc, CHO und C=O), 1248, 1028 (AcO), (Gef.: C, 73·27; H, 9·55. $C_{23}H_{36}O_4$ (M.G. 376·52) Ber.: C, 73·36; H, 9·64%).

(6) *Ringschluss von Ketoaldehyden* (XIX *und* XX)

(i) *Cyclisierung von* XIX. Cyclisierung von XIX sowie XX wurde nach der Angabe[9] durchgeführt. 110·6 mg des oben beschriebenen rohen Ketoaldehyds in 25 cc abs. Xylol und 4 cc einer Lösung von 0·6 cc $(Et)_3N$ und 0·36 cc Eisessig in 10 cc abs. Xylol wurden in ein am einen Ende geschlossenes

Glasrohr eingetragen. Nach starker Abkühlung des einen Endes mit Trockeneis und An (−85°) wurde das Rohr auf unter 1 Torr evakuiert, dann am anderen Ende geschlossen und anschliessend im Xylol-Bad 10 Std. erhitzt.

Nach dem Erkalten wurde die Reaktionslösung mit 2N HCl, dann mit Wasser gewaschen. Die wässrigen Phasen wurden vereinigt und zweimal mit Ae extrahiert.

Die organischen Extrakte ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vak. 122·8 mg kristallinisches Rohprodukt, das aus Ae und Pn umkristallisiert wurde. Die ersten Kristalle (47·6 mg) schmolzen bei 161·5–165°. Aus den Mutterlaugen wurden

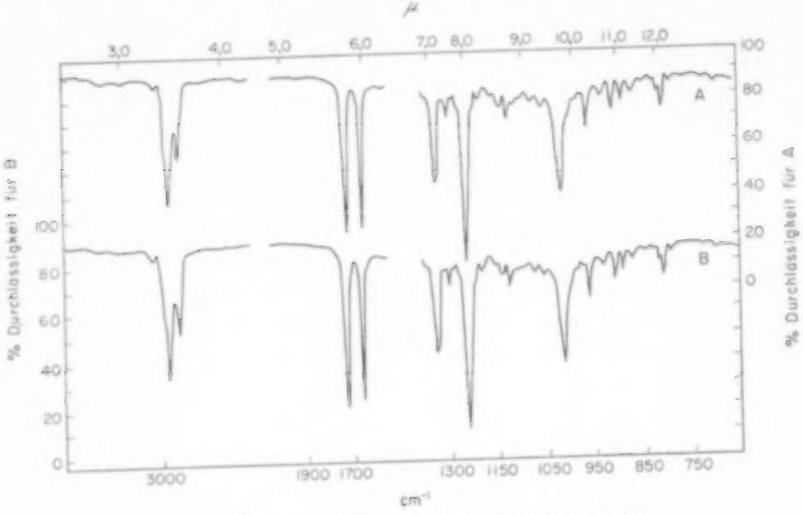


Abb. 1. Infrarot-Spektren (3·5 mg Subst. in 0·2 cm³ CS_2).
A = natürliches d-Δ^{16}-5α-Pregnen-3β-ol-20-on-acetat XXIIIb
B = synthetisches dl-Δ^{16}-5α-Pregnen-3β-ol-20-on-acetat XXIIIa

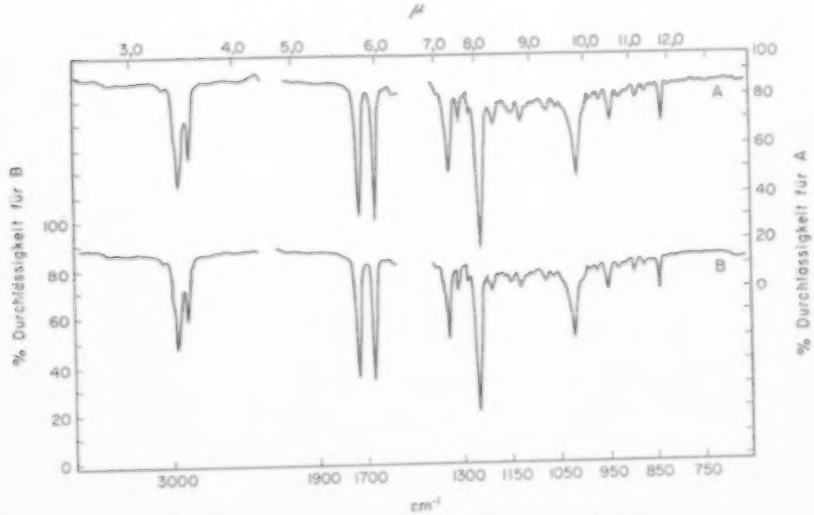


Abb. 2. Infrarot-Spektren (3·5 mg Subst. in 0·2 cm³ CS_2).
A = natürliches d-16-Acetyl-Δ^{16}-androsten-3β-ol-acetat XXIIb
B = synthetisches dl-16-Acetyl-Δ^{16}-androsten-3β-ol-acetat XXIIa

weitere 25·9 mg und 10·5 mg XXIIa vom Schmp. 155–163° bzw. Schmp. 150–158° erhalten. (Insgesamt 84 mg, 80% der Theorie). Weiteres Umkristallisieren aus Me ergab dünne Blätter vom Schmp. 163–165°. Dieses Produkt zeigte in der Mischprobe mit dem Isomer XXIIIa (s. unten) eine merkliche Depression.

Das I.R.-Spektrum dieses dl-16-Acetyl-$\Delta^{16(17)}$-androsten-3β-ol-3-acetates (XXIIa) stimmte mit demjenigen des natürlichen (XXIIb) (über dessen Synthese s. unten) in allen Einzelheiten vollständig überein (Abb. 2). Das U.V.-Spektrum ebenso.

dl-16-Acetyl-$\Delta^{16(17)}$-androsten-3β-ol-3-acetat (XXIIa). Dünne Blätter aus Me, Schmp. 163–165°, I.R. (3·5 mg in 0·2 cc CS_2, vergleiche noch Abb. 2), 3050 $\left(>C=C<^{H}\right)$, 1735 (AcO), 1670 (C=C—C=O), 1238, 1025 (OAc), 849 $\left(>C=C<^{H}\right)$, I.R. ν_{max}^{Nujol} cm^{-1} 3060 $\left(>C=C<^{H}\right)$, 1737 (OAc), 1664 (C=C—C=O), 1594 (scharf, $\left(>C=C<^{H}\right)$, 1248, 1036 (OAc), U.V. λ_{max}^{EtOH} mμ (ε): 241·5 (11500) log ε = 4·06, (Gef.: C, 76·93; H, 9·53. $C_{23}H_{34}O_3$ (M.G. 358·50) Ber.: C, 77·05; H, 9·56%).

Zum papierchromatographischen Vergleich wurde XXIIa mit Na_2CO_3 in wässriger Me-Lösung verseift und das so erhaltene Verseifungsprodukt, dl-16-Acetyl-$\Delta^{16(17)}$-androsten-3β-ol vom Schmp. 225–228°, wurde verwendet.

Die Papierchromatographie des racemischen sowie natürlichen (s. unten) 3β-Alkohols wurde in einem absteigenden System unter Verwendung von Formamid-Aceton (1:2) als stationäre und Toluol-Cyclohexan (1:3) als mobile Phase bei 9·0–11·5° durchgeführt. Zur Erkennung wurde eine Lösung von 20% $SbCl_3$–Chf–Lösung und Beleuchtung von U.V.-Licht angewandt.[12] Die beiden (je 100 γ, Dauer 6·5 Std.) ergaben Flecken vom gleichen *Rf*-Wert von 0·852.

(ii) *Cyclisierung von* XX. 80 mg des oben beschriebenen rohen Ketoaldehydes (XX) in 2 cc abs. Xylol wurden mit 3 cc einer Lösung von 0·6 cc $(Et)_3N$ und 0·36 cc AcOH in 10 cc abs. Xylol gleich wie bei 6-(i) unter vermindertem Druck in einem geschlossenen Glasrohr bei Siedepunkt von Xylol 10 Std. erhitzt.[9]

Die gleiche Aufarbeitung ergab 78·2 mg öliges Produkt, das nach Filtrieren durch eine Al_2O_3 Säule (2 g, Woelm, II) gereinigt wurde. Die mit Pe–Pe–Bzl (1:1) eluierten Anteile ergaben aus Ae und Pn 24·0 mg XXIIIa vom Schmp. 161–166°, das nach weiterem Umkristallisieren aus Me Blätter vom Schmp. 167–169° ergab.

Das I.R.-Spektrum dieses Produktes (XXIIIa) konnte mit demjenigen des natürlichen $\Delta^{16(17)}$-5α-Pregnen-3β-ol-20-on-3-acetat (XXIIIb) vom Schmp. 170·5–171° $[\alpha]_D^{21°}$ = +35·0° (c = 0·879) in Chf, hergestellt nach der Angabe von A. F. B. Cameron *et al.*[10] in allen Einzelheiten vollständig übertragen werden (Abb. 1). Das U.V.-Spektrum war ebenso.

dl-$\Delta^{16(17)}$-5α-Pregnen-3β-ol-20-on-3-acetat (XXIIIa). Blätter aus Me, Schmp. 167–169°, I.R. (3·5 mg in 0·2 cc CS_2, vgl. Abb. 1), 3067 $\left(>C=C<^{H}\right)$, 1735 (OAc), 1670 (C=C—C=O), 1238, 1026 (OAc), 819 $\left(>C=C<^{H}\right)$, U.V. λ_{max}^{EtOH} mμ (ε): 240·5 (9110), log ε = 3·96, (Gef.: C, 77·17; H, 9·50. $C_{23}H_{34}O_3$ (M.G. 358·50) Ber.: C, 77·05; H, 9·56%).

Zum papierchromatographischen Vergleich wurde rohes XXIIIa mit Na_2CO_3 in wässriger Me-Lösung verseift und das rohe Verseifungsprodukt, dl-$\Delta^{16(17)}$-5α-Pregnen-3β-ol-20-on vom Schmp. 178–185°, wurde verwendet.

Die Papierchromatographie des racemischen, sowie natürlichen (s. unten) 3β-Alkohols wurde in einem absteigenden System unter Verwendung von Formamid-Aceton (1:2) als stationäre und Toluol-Cyclohexan (1:3) als mobile Phase bei 9·0–11·5° durchgeführt.

Zur Erkennung wurde eine Lösung von 20% $SbCl_3$–Chf–Lösung und Beleuchtung von U.V.-Licht angewendet.[12] Die beiden (je 100 γ, Dauer 6·5 Std.) ergaben Flecken von fast gleichen *Rf*-Werten von 0·876 (für die racemische) und 0·873 (für die natürliche).

(7) *Abbau des* D-*Homoringes durch Ozonisierung und anschliessende Angliederung des* D-*Ringes*

(i) *Ozonisierung*. 50 mg eines Gemisches XII (Schmp. 122–125°) wurden in 7 cc alkoholfreiem Chf und 3 cc abs. Me gelöst. Unter Abkühlung mit Trockeneis und An (−80°) wurden 150 cc 3·18-proz. (v/v) Ozon enthaltenden Sauerstoffes in einer Geschwindigkeit von 50–60 cc/Min. eingeleitet.

Nach 20-minutigem Stehenlassen bei gleicher Temperatur wurde die Reaktionslösung mit 0·1 cc

[12] R. Neher, *J. Chromat.* **1**, 221 (1958).

Eisessig versetzt und das Ozonid unter Rühren mit 100 mg Zinkstaub zersetzt. Das Reaktionsgut wurde vom Zinkstaub entfernt, im Vak. eingeengt, mit etwas Wasser versetzt und dreimal mit Chf extrahiert. Die Chf-Extrakte wurden mit $NaHCO_3$-Lösung, dann mit Wasser gewaschen und über Na_2SO_4 getrocknet. Eindampfen im Vak. hinterliess 70 mg eines öligen Produktes, das aus einem Gemisch von XIX und XX besteht.

Falls Ozonisierung unter Verwendung von zweifacher Menge von Ozon in reinem Essigester durchgeführt wurde, wurde eine Ketosäure in Nadeln vom Schmp. 226–230° aus An und Ae in einer Ausbeute von ca. 15–20% erhalten.

(ii) *Cyclisierung*. Der oben beschriebene Eindampfrückstand (70 mg) in 1 cc abs. Xylol wurde in Gegenwart von 1·5 cc einer Lösung von 0·6 cc $(Et)_3N$, 0·36 cc AcOH in 10 cc abs. Xylol in einem evakuierten (unter 1 Torr.) geschlossenen Glasrohr auf die Siedetemperatur von Xylol (als Bad) erhitzt. (Siehe 6-(i)).

Die gleiche Aufarbeitung wie bei 6-(i) ergab 47·3 mg eines öligen Produktes, das an 2 g Al_2O_3 (Woelm, Akt. II) chromatographiert wurde.

Die Fraktionen Nr. 4–9 (eluiert mit Pe–Bzl (9:1)–(8:2)) ergaben beim Animpfen der Kriställchen von XXIIIa aus 6-(ii) 2·3 mg rohes XXIIIa, das aus Me umkristallisiert wurde. Blätter vom Schmp. 158–164°. Dieses Produkt erwies sich als identisch mit dem authentischen Präparat von XXIIIa (Mischprobe sowie Vergleich der I.R.-Spektren).

Die Fraktionen Nr. 13–30 (eluiert mit Pe–Bzl (7:3)–(3:7)) ergaben aus Ae–Pn 10·3 mg rohes XXIIa, das aus Ae–Pn nochmals umkristallisiert wurde. Blätter vom Schmp. 162–165°. Dieses Produkt erwies sich als identisch mit dem authentischen Präparat von XXIIa (Mischprobe sowie I.R.-Spektren).

Cyclisierungsversuche mit verschiedenen anderen Mitteln, z.B. HOAc–Piperidin, CH_3ONa und verdünntem wässrigen KOH unter gewöhnlichem Drück ergaben kein identifizierbares Produkt, nur dass bei der Verwendung von HOAc–Piperidin zwei Produkte vom Schmp. 166–169° und vom Schmp. 185–192° in Spuren isoliert wurden, die aber wegen Material-Mangels nicht weiter untersucht wurden.

Ketocarbonsäure. Nadeln aus An und Ae, Schmp. 226–230°, I.R. ν_{max}^{Nujol} cm^{-1} 3150 (breit, COOH), 1733 (AcO), 1716, 1676 (COOH, C=O), 1235, 1034 (AcO), (Gef.: C, 70·41; H, 9·23. $C_{25}H_{36}O_5$ (M.G. 392·52) Ber.: C, 70·37; H, 9·24%).

III. *Herstellung von Vergleichsubstanzen aus den natürlichen Steroiden*

(1) $\Delta^{16(17)}$-5α-*Pregnen*-3β-*ol*-20-*on*-3-*acetat* (XXIIIb) *und dessen Verseifungsprodukt*

Diese Substanz wurde nach der Angabe von A. F. B. Cameron *et al.*[11] aus Hecogeninacetat hergestellt.

$\Delta^{16(17)}$-5α-Pregnen-3β-ol-20-on-3-acetat (XXIIIb). Säulen aus An und Ae, Schmp. 170–171° (In der Literatur: Schmp. 165–167°), $[\alpha]_D^{21°} = +35·0°$ (±3°) $c = 0·879$ (Chf), I.R. (3·5 mg in 0·2 cc CS_2, vgl. Abb. 1) 3065 $\left(>C=C<^{H}\right)$, 1735 (OAc), 1670 (C—C—C=O), 1239, 1026 (OAc), 819 $\left(>C=C<^{H}\right)$, U.V. λ_{max}^{EtOH} mμ (ε): 240·5 (9230), log ε = 3·97, (Gef.: C, 76·87; H, 9·49. $C_{23}H_{34}O_3$ (M.G. 358·50) Ber.: 77·05; H, 9·56%).

Verseifung dieses Produktes wurde durch Kochen mit Na_2CO_3 in wässriger Me-Lösung bewirkt. Nadeln aus An und Ae, Schmp. 202–207°. Dieses Verseifungsprodukt wurde zur Papierchromatographie benutzt (s. oben).

(2) 17β-*Methyl*-D-*homo*-*androstan*-3β,17α,17aα-*triol*-3-*acetat* (XVb) *und dessen Verseifungsprodukt*

Das Ausgangsmaterial (XVII) wurde nach der Angabe[8] aus 5α-Pregnan-3β,17α-diol-20-on-3-acetat (XVIII) durch D-Homoannulierung hergestellt.

XVII wurde in Anlehnung an die Wendler'sche Methode[7] mit Al-isopropoxyd reduziert. 120 mg XVII wurden in 10 cc abs. Toluol gelöst und durch Abdestillieren von 5 cc azeotropem Destillat von dem anwesenden Wasser befreit. Darin wurden 120 mg Al-isopropoxyd eingetragen und die Reaktionslösung 2 Std. unter Rückfluss gekocht.

Nach dem Erkalten wurde etwas Wasser zugegeben und die wässrige Phase dreimal mit einer Mischung von Ae und Chf (1:1) extrahiert. Die organischen Auszüge ergaben nach Waschen mit Wasser, Trocknen über Na_2SO_4 und Eindampfen im Vak. 118·4 mg kristallinisches Produkt, das an 4 g Al_2O_3 (Woelm, Akt. II) chromatographiert wurde.

Die mit Bzl–Chf (8:2)–(1:1) eluierten Fraktionen ergaben aus An und Pn oder Chf und Pn 75·1 mg rohes XVb in Nadeln (Busch) vom Schmp. 183–186°, das nach nochmaligem Umkristallisieren bei 186–188° schmolz.

I.R.-Spektrum (Chf) dieses Produktes war mit demjenigen der dl-Substanz XVa in allen Einzelheiten identisch, wonach die α-Konfiguration der Hydroxylgruppe am C_{17a} sicher festgestellt werden konnte.

Die mit Bzl–Chf (3:7)–Chf–Me (98:2) eluierten Fraktionen ergaben aus Me (wenig), Chf und Pn 22·5 mg Kristalle vom Schmp. 250–252°, die nach weiterem Umkristallisieren bei 249–251° schmolzen. Dieses Produkt stellte nach der Analyse, sowie dem I.R.-Spektrum ein Verseifungsprodukt von XVb dar.

17β-Methyl-D-homo-androstan-3β,17α,17aα-triol-3-acetat (XVb). Nadeln aus Chf und Pn, Schmp. 186–188°, I.R. ν_{max}^{Chf} cm^{-1} 3560, 3520–3300 (OH), 1726, 1250, 1027 (OAc), $[\alpha]_D^{21°} = -0·7° (\pm 5°)$ C = 0·582 (Chf), (Gef.: C, 72·51; H, 10·00. $C_{22}H_{38}O_4$ (M.G. 378·53) Ber.: C, 72·97; H, 10·12%).

17β-Methyl-D-homoandrostan-3β,17α,17aα-triol. Säulen aus Me, Chf und Ae, Schmp. 249–251°, I.R. ν_{max}^{Nujol} cm^{-1} 3320–3440 (OH), 1047, 1037 (C—O), (getrocknet im Vak. über P_2O_5 bei 150° bis konstantem Gewicht) (Gef.: C, 74·54; H, 10·66. $C_{21}H_{36}O_3$ (M.G. 336·50) Ber.: C, 74·95; H, 10·78%).

(3) 16-*Acetyl*-$\Delta^{16(17)}$-*androsten*-3β-*ol*-3-*acetat* (XXIIb) *aus* (XVb)

60 mg (0·156 m. Mol) XVb wurden in 1 cc Me und 0·3 cc Dioxan gelöst. Dazu wurde eine Lösung von 50 mg (0·219 m. Mol) $HJO_4.2H_2O$ in 1 cc Wasser zugefügt und die Reaktionslösung wurde 1·5 Std. bei Raumtemperatur stehen gelassen. Die gleiche Aufarbeitung wie bei 6-(i) ergab 59·2 mg Eindampfrückstand, der sofort in 1 cc Xylol (wasserfrei) gelöst und gleich wie bei 6-(i) in Gegenwart von 1·5 cc einer Lösung von 0·6 cc $(Et)_3N$, 0·36 cc AcOH in 10 cc Xylol in einem evakuierten geschlossenen Glasrohr 10 Std. in siedendem Xylol erhitzt wurde.

Die Aufarbeitung ergab 65·2 mg öliges Produkt, das an 2 g Al_2O_3 (Woelm, Akt. II) chromatographiert wurde.

Die Fraktionen (eluiert mit Pe–Bzl (9:1)–Bzl) ergaben 35·9 mg rohes XXIIb vom Schmp. 147–149°, das aus Ae und Pn oder aus Me umkristallisiert wurde. Spindeln vom Schmp. 149–151°.

Dessen I.R.-sowie U.V.-Spektrum war, wie oben beschrieben 6-(i), mit demjenigen des totalsynthetisch hergestellten Präparates (XXIIa) vollständig identisch.

16-Acetyl-$\Delta^{16(17)}$-androsten-3β-ol-3-acetat (XXIIb). Spindeln aus Me, Schmp. 149–151°, $[\alpha]_D^{21°} = -55·0° (\pm 3°)$ $c = 0·889$ (Chf), I.R. (3·5 mg in 0·2 cc CS_2, vergleiche noch Abb. 2) 3050 $\left(>C{=}C{<}^{H}\right)$, 1735 (AcO), 1670 (C=C—C=O), 1238, 1024 (OAc), 848 $\left(>C{=}C{<}^{H}\right)$, I.R. ν_{max}^{Chf} cm^{-1} 3040 $\left(>C{=}C{<}^{H}\right)$, 1725 (AcO), 1663 (C=C—C=O), 1590 (scharf, $>C{=}C{<}^{H}$), 1250, 1023 (AcO), 854 $\left(>C{=}C{<}^{H}\right)$, U.V. λ_{max}^{EtOH} mμ (ε): 241·5 (11200), log ε = 4·05, (Gef.: C, 77·45; H, 9·69. $C_{23}H_{34}O_3$ (M.G. 358·50) Ber.: C, 77·05; H, 9·56%).

Die Verseifung dieses Produktes wurde mit Na_2CO_3 in wässriger Me-Lösung durch Kochen (1 Std.) bewirkt. Prismen aus Chf (wenig), An und Ae, Schmp. 205–209°. Dieses Verseifungsprodukt wurde zur Papierchromatographie benutzt (s. oben).

Für die Unterstützung bei dieser Arbeit und die wertvolle Diskussion darüber danken wir Herrn Prof. E. Ochiai herzlichst. Wir danken auch den Herren unseres Mikrolaboratoriums für die Analysen daten und Herrn Dr. T. Kubota und Herrn Y. Matsui für die optischen Daten.

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STUDIES ON SESQUITERPENOIDS—III[1]

SOME DERIVATIVES OF GUAIOL[2]

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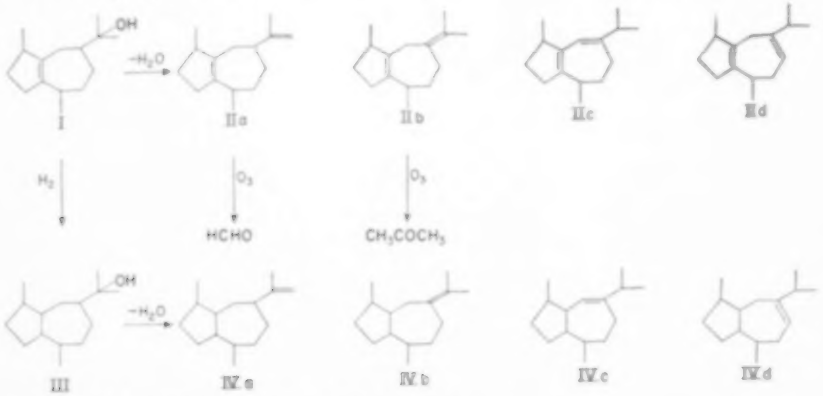
Abstract—When guaiol was dehydrated with such reagents as thionyl chloride–pyridine, phosphorus oxychloride–pyridine or potassium bisulphate, the isopropenyl derivative (IIa) was always obtained as a main product, together with a small amount of IIb. Catalytic reduction of IIa in alkaline media afforded α-dihydroguaiene (V), while in neutral or acid media it gave a mixture of V and its double bond isomer (VI).

THE structure of guaiol, $C_{15}H_{26}O$ (I), a well known sesquiterpene alcohol isolated from the Guaiacum wood oil,[3] was completely established by Plattner and his collaborators.[4] As the hydroxyl group in guaiol is a tertiary one, guaiol is easily dehydrated by means of the various dehydrating reagents and this reaction has been studied by many authors.[5,6] In this reaction only a mixture of the unsaturated substances (IIb, IIc and IId),[5] so-called "guaiene", b.p. 128–130°/12 mm, n_D^{20} 1·5022, $[\alpha]_D$ −16·8°, was obtained, but no precise structural elucidation of each compound has been given in the literature.

Plattner and his co-workers[4] also examined on the dehydration reaction of dihydroguaiol (III), which was obtained by the catalytic hydrogenation of guaiol with Raney nickel under high pressure, and a mixture of the unsaturated hydrocarbons, designated



[1] Part II: Hitoshi Minato, *Chem. Pharm. Bull.* To be published.
[2] An outline of this paper was reported at the I.U.P.A.C. Symposium on the Chemistry of Natural Products in Sydney, Australia, 23 August 1960.
[3] Guaiol was purchased from Polak & Schwarz's Essence-Fabriken, New York.
[4] [a] A. St. Pfau and Pl. A. Plattner, *Helv. Chim. Acta* **19**, 858 (1936); [b] Pl. A. Plattner and L. Lemay, *Ibid.* **23**, 897 (1940); [c] Pl. A. Plattner and G. Magyar, *Ibid.* **24**, 191 (1941); [d] *Ibid.* **25**, 581 (1942); [e] Pl. A. Plattner, *Ibid.* **24**, 283E (1941).
[5] O. Wallach, *Liebigs Ann.* **279**, 395 (1894); A. Gandurin, *Ber. Dtsch. Chem. Ges.* **41**, 4359 (1908); L. Ruzicka, S. Pontalti and Fr. Balas, *Helv. Chim. Acta* **6**, 855 (1923).
[6] J. Pliva and F. Šorm, *Coll. Czech. Chem. Comm.* **14**, 274 (1949).

as "dihydroguaiene", was obtained. They assumed that the so-called "dihydroguaiene" is a mixture of IVb, IVc and IVd from the results of ozonolysis of this "dihydroguaiene".

In the present paper, we wish to report the results of the dehydration reaction of guaiol with various reagents, and furthermore, some interesting findings on the reduction products of "α-guaiene" (IIa).

(1) *Dehydration reaction of guaiol*

When guaiol was dehydrated with thionyl chloride in pyridine at 60° and the products were carefully fractionated* and the following fractions were obtained (*cf.* Table 1).

TABLE 1. DEHYDRATION PRODUCTS OF GUAIOL

Entry no.	Fraction no. on distill.	Boiling point	n_D^{20}	Ratio of peak height at 9·3 min : 11·3 min	$[\alpha]_D$	Yield (ml)
		Dehydration with $SOCl_2$-pyridine				
1	1	78–79°/2·5 mm	1·4958	1 : 0	−64·5°	2·5
2	2	78–79°/2·5 mm	1·4958	1 : 0·034	—	5·7
3	3	78–79°/2·5 mm	1·4963	1 : 0·056	—	18·3
4	4	79–81°/2·5 mm	1·4969	1 : 0·107	−55·1°	5·0
5	5	81–83°/3 mm	1·5045	1 : 0·477	—	5·7
6	6[a]	87–92°/3 mm	1·5053	1 : 0·495	—	11·9
		Dehydration with $POCl_3$-pyridine				
7	—	82–84°/3 mm	1·4991	1 : 0·262	—	—
		Dehydration with $KHSO_4$				
8	1	96–97°/3·5 mm	—	1 : 0·475 : 0·296[b]	—	6·54
9	2	97–98°/3·5 mm	—	1 : 0·775 : 0·552[b]	—	20·63
10	3	98–100°/3·5 mm	—	1 : 1·480 : 1·06[b]	—	11·36
11	entry 7 + 10 1 : 1	—	—	1 : 0·559 : 0·285[b]	—	—

[a] Fraction no. 6 was the residue of the distillation, and redistillated. This fraction showed the shoulder at 250 mμ (ε 780) in U.V. and the weak absorption band at 3491 cm^{-1} (OH) and 1709 cm^{-1} (C=O) in I.R. spectra.

[b] Ratio of peak height at 9·3 min : 11·3 min : 12·3 min.

Each fraction was examined by gas chromatography and spectroscopic methods. As shown in Table 1, Fraction no. 1 showed only one peak at the retention time of 9·3 min in the gas chromatogram, and Fractions nos. 2–5 all showed two peaks at retention times of 9·3 min and 11·3 min. The infra-red spectrum of each fraction showed three absorption bands at 3100 (C—H), 1645 (C=C) and 884 cm^{-1} corresponding to the $>C=CH_2$ type double bond. The ratio of the peak height at 9·3 min and 11·3 min in the gas chromatogram and the intensity of each three absorption bands in the infra-red spectrum both gradually decreased according to the increase in the fraction number.

Purified Fraction no. 1 showed absorption bands at 3100, 1645 and 884 cm^{-1} corresponding to the isopropenyl functions in the infra-red spectrum, but no other characteristic band corresponding to the conjugated diene or the trisubstituted double

* See experimental.

bond in both the infra-red and the ultra-violet spectrum, and showed the following physical data: b.p. 78–79°/2·5 mm, n_D^{20} 1·4958, d_4^{20} 0·8999, $[\alpha]_D^{19}$ −64·5°. Formaline was obtained as its dimedon-derivative when it was ozonized under usual conditions. On the other hand, this substance was easily hydrogenated to a dihydro derivative and the reduction product no longer showed an absorption band corresponding to the isopropenyl functions. Although the isopropenyl type dehydration product of guaiol was hitherto unknown in the literature,[7] the constitution of this substance has been shown to be IIa from the above-mentioned results, and the name "α-guaiene" will be given to this substance.

As the substance having a peak at a retention time of 11·3 min afforded acetone by ozonolysis and the infra-red and the ultra-violet spectrum of this substance showed no band corresponding to the conjugated diene system or the trisubstituted double bond, this substance was assumed to be IIb, the structure of which has been already reported by Plattner and other authors.

The results of the dehydration reaction of guaiol with phosphorus oxychloride in pyridine were very similar to those obtained when thionyl chloride was used (*cf.* Table 1). When potassium bisulphate was used as a dehydrating reagent, it gave more complicated results. In this case, each fraction showed three peaks at retention times of 9·3 min, 11·3 min and 12·3 min in the gas chromatogram (*cf.* Table 1). It has been proved that the former two peaks are identical with those of Fraction no. 2, the thionyl chloride–pyridine dehydration product, when a mixed sample (entry 11 on Table 1) was examined by gas chromatography (*cf.* Fig. 1).

Although the infra-red spectrum of each fraction was similar to that of IIa, the intensity of each three absorption bands corresponding to the isopropenyl functions all decreased. Moreover, each fraction showed two more new weak bands at 865 and 834 cm^{-1} in the infra-red spectrum.

From these observations, the substance having a peak at a retention time of 12·3 min may be a new substance, but it was impossible to obtain this substance in a pure state. However, it may be concluded that when potassium bisulphate was used the dehydration product of guaiol mainly consisted of α-guaiene and IIb together with a small amount of the substance of an unknown structure.

(2) *Catalytic hydrogenation of α-guaiene*

On catalytic hydrogenation of α-guaiene, 1 mole of hydrogen was taken up smoothly and a reduction product corresponding to the dihydro derivative was obtained. When α-guaiene was reduced in ethyl acetate with palladium charcoal as a catalyst under usual conditions, it gave a colourless mobile oil, b.p. 79·5°/2 mm, n_D^{20} 1·4889. The analytical values of this oil are in good agreement with the formula $C_{15}H_{26}$ and all the absorption bands corresponding to the isopropenyl group disappeared in the infra-red spectrum. However, this product showed a considerable positive rotatory power, $[\alpha]_D^{21}$ +52·9°, in spite of the fact that the starting material showed a negative rotation, $[\alpha]_D^{19}$ −64·5°. These results suggest the migration of the double bond between C—1 and C—5 in α-guaiene. We now questioned the purity of the reduced oil and examined it by gas chromatography (cf. Table 2). This oil showed two peaks at retention times of 8·8 min and 11·25 min in the gas chromatogram and it

[7] G. Chiurdoglu and M. Descamps, *Tetrahedron* **8**, 271 (1960). It was described that guaiol (I) was dehydrated to give IIa with an acid catalyst, but its details have not been reported in this paper.

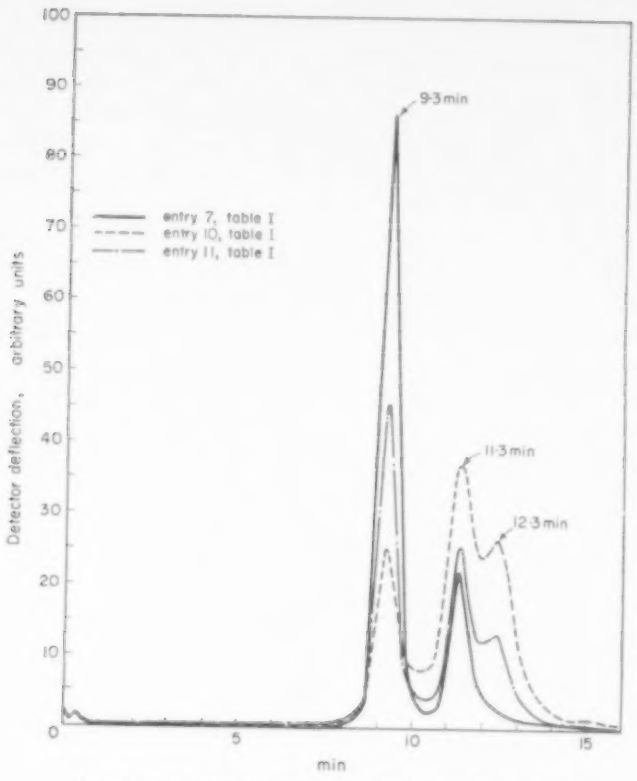
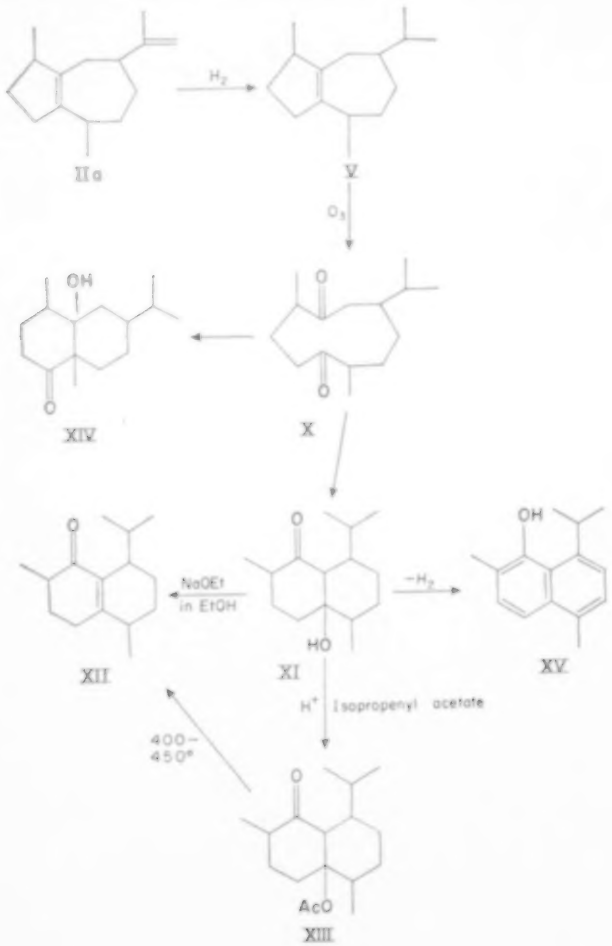


FIG. 1. Gas chromatogram of dehydration products of guaiol.

TABLE 2. HYDROGENATION PRODUCTS OF α-GUAIENE

Entry no.	Catalyst	Solvent	Ratio of peak height at 8·8 min : 11·25 min	$[\alpha]_D$	n_D^{20}
1	10% Pd/C	Ethylacetate	1 : 1·43	+52·9°	1·4889
2	10% Pd/C	Ethylacetate	1 : 1·59	+59·3°	1·4905
3	10% Pd/C	Acetic acid	1 : 1·37	—	—
4	5% Pd/$CaCO_3$	Acetone	1 : 1·56	—	—
5	Raney Ni	Xylene	1 : 0·9	—	—
6	Raney Ni	Dioxane-MeOH NaOH	1 : 0	−30·2°	1·4879
7	10% Pd/C	Dioxane-MeOH KOH	1 : 0	—	—

indicated that the reduced oily substance is a mixture of two components, as expected. By gas chromatographic examination it was proved that in all cases when reduction of α-guaiene was carried out under acid or neutral conditions, it afforded a mixture of two components as in the above-mentioned case, whereas under alkaline conditions it gave only one substance and this showed a peak at the retention time of 8·8 min only. The purified reduction product, obtained under alkaline conditions, showed the following constants, b.p. 104–106°/5 mm, n_D^{20} 1·4879, d_4^{20} 0·8877, $[\alpha]_D^{23}$ −30·2°, and the analytical data of this substance coincides well with those of dihydroguaiene. The infra-red spectrum of this compound no longer showed an absorption band corresponding to the isopropenyl group but a strong band at 1664 cm^{-1} corresponding to the C=C double bond was observed in the Raman spectrum. This compound is considered to be the anticipated dihydroguaiene represented by structure V, since it may be expected that the double bond migration will not occur under such alkaline conditions and the value of the optical rotation is also reasonable. This structure was further confirmed chemically as mentioned below.



When an unpurified oily diketone (X), ν C=O 1710 cm^{-1}, obtained by ozonolysis of the afore-mentioned reduction product, was treated with acetic acid under reflux for 1 hr, or with 0·1 N solution of sodium methoxide in methanol under reflux for 1 hr, or by heating at 190–200° without a solvent, in all cases it afforded the same crystalline ketol (XI), $C_{15}H_{26}O_2$, m.p. 147–149°, $[\alpha]_D^{22}$ +88·6°. Although the infra-red spectrum of this substance showed the presence of the hydroxyl group, ν O—H 3406 cm^{-1}, it gave no acetate under usual conditions but a monoacetate (XIII), m.p. 130–131°, when it was treated with isopropenyl acetate in the presence of *p*-toluenesulphonic acid. In spite of the presence of an absorption band at 1692 cm^{-1} corresponding to the six-membered ring ketone in the infra-red spectrum, it gave neither a semicarbazone nor a 2,4-dinitrophenylhydrazone when reacted in the usual manner. From these observations it was assumed that the hydroxyl group in this ketol is a tertiary one and that the position of the ketone is very sterically hindered. On treatment with 5 per cent sodium ethoxide in ethanol under reflux for 4 hr, the ketol underwent dehydration to produce α,β-unsaturated ketone (XII), b.p. 115–116°/2 mm, whose ultra-violet spectrum showed a maximum at 247·5 mμ (ε 11·200) and the infra-red spectrum also showed absorption bands at 1665 and 1622 cm^{-1} indicating the presence of the α,β-unsaturated ketone. This α,β-unsaturated ketone was also obtained by pyrolysis of the acetoxyketone (XIII) at 400–450°. Since the structure XIV for this ketol was excluded from the two possible alternatives by virtue of the results of the above-mentioned dehydration reaction, the structure of this compound was assumed to be 10-hydroxy-2,5-dimethyl-8-isopropyldecalone-1 (XI). In order to confirm this assumption the foregoing ketol was subjected to dehydrogenation with palladium charcoal at 300–350°, and the anticipated 2,5-dimethyl-8-isopropyl-1-naphthol (XV) was isolated as the sole product. The identity of the dehydrogenation product was evidenced as its picrate, m.p. 135–136° and the trinitrobenzene adduct, m.p. 136–137°. The results of the ultra-violet and the infra-red spectra also satisfied the structure of XV (*cf.* Fig. 2).

Now as the correctness of the assumed structure V for the reduction product of α-guaiene under alkaline conditions, was proved from these experimental results, we wish to give the name "α-dihydroguaiene"* to this compound.

Next we examined another reduction product of α-guaiene having a peak at a retention time of 11·25 min in the gas chromatogram. As this compound is considered to be a double bond isomer of α-dihydroguaiene (V), the possible structures of the isomer will be represented as VI-IX. Although it was impossible to obtain this isomer in a pure state, we finally obtained an isomer rich fraction from the Podbielniak column distillation of the dihydroguaiene mixture. This fraction, b.p. 87–89°/2·5 mm, showed two peaks at retention times of 8·8 min and 11·25 min in the gas chromatogram and the ratio of the peak height at 8·8 min to 11·25 min of this fraction is about 1 : 3. Since the Raman spectrum of this fraction showed a strong double bond absorption at 1664 cm^{-1} but its infra-red spectrum showed no band corresponding to the trisubstituted double bond, numbers VIII and IX out of the four possible structures are therefore excluded for this compound.

Ozone oxidation of the above-mentioned fraction gave an oily substance (XVI),

* In 1908, Gandurin[5] also reported a substance corresponding to the dihydro derivative of guaiene, which was obtained from guaiol by the action of zinc dust at 220°. This substance, b.p. 122°/11 mm, n_D^{20} 1·4982, d_4^{20} 0·8914, $[\alpha]_D^{19.5}$ −26·65°, seems to be identical with the α-dihydroguaiene obtained by us.

b.p. 115–120°/1·6 mm. This product showed negative results to Fehling's and Tollen's reagents, and showed absorption bands at 1709 (C=O) and 1410 cm^{-1} (—CH_2—CO—). The results of the spectroscopic examination and the aldehyde-test showed that this

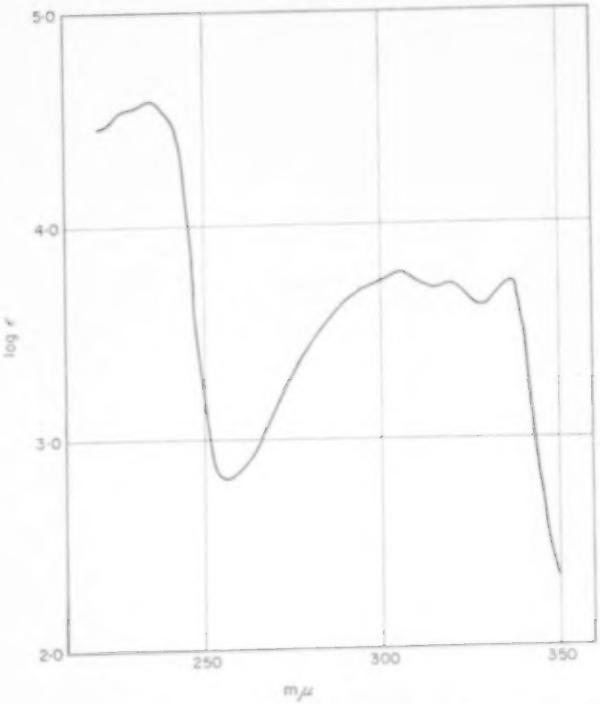
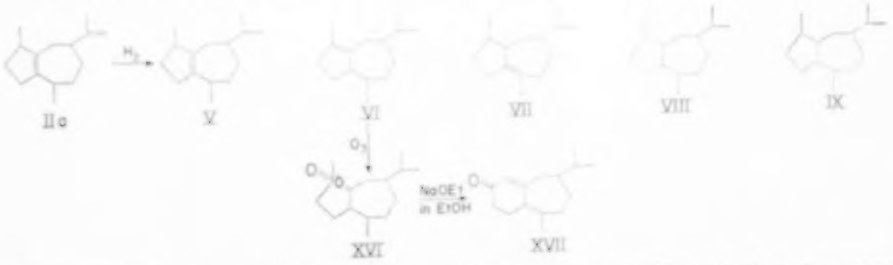


FIG. 2. Absorption max (mμ) and log ε of 2,5-dimethyl-8-isopropylnaphthol-1 (XV). (In ethanol.)

oxidation product has neither a five-membered ring ketone nor an aldehyde function. Since structures VII, VIII and IX for this isomer do not satisfy the above-mentioned results, structure VI is the most likely. An α,β-unsaturated ketone (XVII) having an

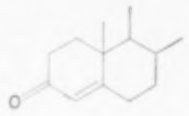


absorption band at 244·5 mμ in the ultra-violet spectrum was formed when the ozone oxidation product was treated with an alkali (*cf.* Chart 3). The absorption band at 244·5 mμ is in agreement with the band of the type XVII[8,9] and this result also supports

[8] Calculated value of 4-ene-3-one in steroids is 244 mμ. L. F. Fieser and M. Fieser, *Steroids* p. 19. Reinhold, New York (1959).
[9] M. Palmade and G. Ourisson, *Bull. Soc. Chim. Fr.* 886 (1958).

the assumed structure VI for the isomeric dihydroguaiene. It is interesting to note that the double bond migration from the C—1 : C—5 position occurs under such mild conditions.

Stereochemical examination of the decalolone derivative (XI) and further confirmation of the structure of VI are now under investigation in our laboratory.



EXPERIMENTAL

The Shimadzu G.O-IA Vapor Fractomer was used for all gas chromatographic determinations. For the analysis of products, a column, 3 m in length with a 6 mm i.d., consisting of Silicone 550 on fire brick (20 to 40 mesh) was operated at 200° with a flow rate of 100 ml/min of hydrogen.

All melting points were measured by use of Kofler block ("mono-scope" Hans Bock Co. Ltd., Frankfurt am Main, Germany) and corrected.

α-*Guaiene* (IIa)

(1) *Dehydration of guaiol* (I) *with thionyl chloride–pyridine.* Thionyl chloride (47·7 g, 1·38 equiv.) was added dropwise to a solution of guaiol (64·5 g) in pyridine (300 ml) with stirring at 5–10° for 30 min. After the mixture was left 30 min at 4–5°, heated at 55–65° for 1 hr with protection from moisture. Then the mixture was evaporated into about one-fifth *in vacuo*, poured onto a mixture (200 g) of ice and water, and extracted with ether. The ether extract was washed with 2 N H_2SO_4, water and 2 N Na_2CO_3, dried (Na_2SO_4) and evaporated giving a deep brown soiled oil (56 g). The residue was purified to some extent by distillation, and moreover redistilled through a column, 300 mm high with a 10 mm i.d., packed with single turn helices no. 20 (stainless steel), to furnish six fractions (see Table 1).

Fraction 1 was pure α-guaiene (IIa), a colourless mobile oil, b.p. 78–79°/2·5 mm, n_D^{20} 1·4958, d_4^{20} 0·8999, $[\alpha]_D^{19}$ −64·5° (±2°) (c 3·584, dioxane), ν_{max}^{film} 3100, 1645 and 884 cm^{-1}; retention time, 9·3 min. (Found: C, 87·65; H, 11·76. $C_{15}H_{24}$ requires: C, 88·16; H, 11·84%).

In Fractions 2–5, the data of gas chromatography indicated the presence of IIa and IIb, retention times of which are 9·3 and 11·3 min respectively, and the ratio of these peak heights was noted in Table 1. Fraction 6 was the residue of rectification, and redistilled by usual manner. This fraction showed the shoulder at 250 mμ (ε 780) in the ultra-violet and weak absorption bands at 3491 and 1709 cm^{-1} in the infra-red spectrum, whereas in the gas chromatogram it showed no peaks except those at 9·3 and 11·3 min.

(2) *Dehydration of guaiol* (I) *with phosphorous oxychloride–pyridine.* $POCl_3$ (8·8 g, 1·2 equiv.) was added dropwise to a solution of guaiol (10·6 g) in pyridine (90 ml) with stirring at room temp. The mixture was refluxed for 2 hr, and worked up as described above.

(3) *Dehydration of guaiol* (I) *with potassium bisulphate.* A mixture of guaiol (46·5 g) and fused potassium bisulphate (28·5 g) was heated at 160–165° under slightly reduced pressure for 30 min, and worked up as described in the case of (1).

Ozonolyses of α-*guaiene* (IIa) *and guaiene mixture*

(a) IIa (1·02 g) in carbontetrachloride (15 ml) was ozonized at 0°, and the issuing gas was passed through two bottles containing water (A) (ice bath). The solution of ozonide was decomposed by heating with water (40 ml), and then an alcohol solution of dimedone was added to a half of this aqueous solution (B). The remaining solution of ozonide was distilled and the volatile ketone was trapped in aqueous *p*-nitrophenylhydrazine acetate solution (C). The presence of formaldehyde in the aqueous solution (A) was proved as its dimedone-derivative (223 mg). As this dimedone-derivative (50 mg) also was obtained from (B), the total yield was 323 mg (22·1%). Formaldehyde-dimedone derivative was recrystallized from ethanol as colourless needles, m.p. 191–192°, undepressed on admixture with the authentic sample. From (C) acetone-*p*-nitrophenylhydrazone was not obtained at all.

(b) Guaiene (1·02 g, the ratio of peak heights at 9·3 and 11·3 min was 1 : 0·17) was ozonized as described above. The total yield of the formaldehyde-dimedone derivative was 252 mg (17·3%). Acetone-*p*-nitrophenylhydrazone was recrystallized from ethanol as yellowish orange needles (98 mg, 10·2%), m.p. 147–149°, undepressed on admixture with the authentic sample.

Dihydro-α-guaiene (V)

(1) *Hydrogenation of* IIa *under alkaline condition.* Raney Ni catalyst (35 g) was added to a solution of IIa (18·7 g) in dioxane (200 ml) and methanol (50 ml) containing 2 ml of 50% aqueous sodium hydroxide. The mixture was reduced by catalytic hydrogenation at room temp and atmospheric pressure. When 2100 ml (1·05 moles) of hydrogen had been absorbed, the reaction stopped, and the catalyst and the solvent were removed. The residue was extracted with ether, washed with water, dried (Na_2SO_4) and evaporated leaving a colourless oil (18·5 g). This oil was distilled at 79–80°/2 mm (or 104–106°/5 mm) giving V (17·9 g), n_D^{20} 1·4879, d_4^{20} 0·8877, $[\alpha]_D^{22}$ −30·2° (±2°) (c 1·866, dioxane). V showed no characteristic absorption band in the infra-red spectrum, and showed only one peak at a retention time, 8·8 min in the gas chromatogram (see Table 2). (Found: C, 87·14; H, 12·70. $C_{15}H_{26}$ requires: C, 87·30; H, 12·70%).

(2) *Hydrogenation of* IIa *under neutral condition.* Ten per cent palladized charcoal catalyst (630 mg) was added to a solution of IIa (6·27 g) in ethyl acetate (60 ml). The mixture was reduced by catalytic hydrogenation at room temp and atmospheric pressure. When 858 ml (1·13 moles) of hydrogen had been absorbed, the reaction stopped, and the mixture was worked up as described above leaving a colourless oil (6·0 g). This oil was distilled at 79·5°/2 mm giving a mixture (4·5 g) of V and VI, n_D^{20} 1·4889, $[\alpha]_D^{21}$ −52·9° (c 8·80, dioxane), which was almost identical with V in the infra-red spectrum. The data of gas chromatography indicated two peaks at retention times of 8·8 and 11·25 min and the ratio of the peak heights was 1 : 1·43.

(3) *Hydrogenation of* IIa *under acid condition.* A mixture of 10% palladized charcoal catalyst (100 mg) and IIa (1 g) in glacial acetic acid (20 ml) was reduced catalytically at room temp and atmospheric pressure yielding a colourless oil (856 mg). This oil was distilled at 90–92·5°/3·5 mm giving a mixture (640 mg) of V and VI. The ratio of the peak heights at 8·8 and 11·25 min was 1 : 1·37.

Ozonolysis of α-dihydroguaiene (V)

A solution of V (6 g) in ethyl acetate (20 ml) was treated at −75° with oxygen–ozone stream containing 3 to 3·2 wt.% ozone. After 1·5 molar equivalents of ozone was passed through the solution, it coloured blue, and then the solution was allowed to stand at −75° for 30 min. Subsequently, the solvent was removed under high vacuum at room temp. To a solution of the residue in acetic acid (30 ml) was added zinc dust (2 g) in small portions with stirring (ice bath), during which time the temp was kept below 20°, and then the mixture was negative to colour test for zinc iodide–starch paper. The solution was evaporated *in vacuo*, extracted with ether, washed with water, saturated potassium bicarbonate solution and water, dried (Na_2SO_4) and evaporated giving a yellow oil (X) (5·7 g), ν_{max}^{film} 1710 cm^{-1}.

10-*Hydroxy*-2,5-*dimethyl*-8-*isopropyldeculone*-1 (XI)

(1) A solution of crude X (4·9 g) in glacial acetic acid (25 ml) was refluxed for 1 hr. The solution was evaporated, extracted with ether, washed with water, saturated potassium bicarbonate solution and water and dried (Na_2SO_4). The residue obtained upon removal of the solvent was crystallized with the addition of petroleum ether or ether. Recrystallization from petroleum ether–ether gave XI as fine colourless needles (1·91 g, 38·6%), m.p. 147–149°, $[\alpha]_D^{22}$ +88·6° (±2°) (c 0·979, ethanol), ν_{max}^{Nujol} 3406, 1692, 1012 and 998 $cm.^{-1}$ (Found: C, 75·25; H, 10·88. $C_{15}H_{26}O_2$ requires: C, 75·58; H, 11·00%).

(2) Crude X (835 mg) was heated at 190–210° (oil bath) for 1·5 hr. The remaining brown thick oil was recrystallized with the addition of ether giving colourless needles (223 mg, 26·8%), m.p. 146–147°. The crystalline substance was shown to be XI by mixed melting point (146–148°).

(3) To a solution of Na (23 mg) in methanol (10 ml) was added crude X (238 mg). The mixture was refluxed in nitrogen atmosphere for 40 min, then evaporated *in vacuo*, extracted with ether, washed with water, dried (Na_2SO_4) and evaporated giving colourless needles (53 mg), m.p. 147°, $[\alpha]_D^{21}$ +92·9° (±4°) (c 0·692, ethanol), which was shown to be XI by mixed melting point (147–148°).

Treatment of XI *with glacial acetic acid*

A solution of XI (134 mg) in glacial acetic acid (3 ml) was refluxed for 1 hr. The mixture was evaporated *in vacuo*, extracted with ether, washed with water, saturated potassium bicarbonate solution and water, dried (Na_2SO_4) and evaporated leaving XI (125 mg).

Treatment of XI *with* 0·1 N *sodium methoxide in methanol*

To a solution of Na (23 mg) in methanol (10 ml) was added XI (199 mg). The mixture was refluxed in nitrogen atmosphere for 2 hr. This mixture was worked up as described already giving XI (155 mg).

Dehydrogenation of 10-*hydroxy*-2,5-*dimethyl*-8-*isopropyldecalone*-1 (XI)

A mixture of XI (500 mg) and 20% palladized charcoal (250 mg) was heated at 300–350° (bath temp) for 2 hr, during which time ca. 100 ml (1·9 moles) of hydrogen was generated. The residue was extracted with petroleum ether and washed with Claisen's solution. The acid fraction gave a pale yellow oil (16 mg) and from the neutral fraction a pale yellow oil (370 mg) was obtained. This neutral oil was distilled giving a colourless oil (270 mg), b.p. 112–114°/0·2 mm, and gave a picrate as brownish red needles, m.p. 135–136° (recrystallized from ethanol three times) which was identical with a picrate of 2,5-dimethyl-8-isopropyl-naphthol-1 (XV).[4d] (Found: C, 57·12; H, 4·57; N, 9·67. $C_{15}H_{18}O \cdot C_6H_3O_7N_3$ requires: C, 56·88; H, 4·77; N, 9·48%).

The picrate (160 mg) was dissolved in petroleum ether–ether (1:1), and filtered through a column of alumina (3 g). From the filtrate was obtained a colourless oil (58 mg). To a solution of this oil in ethanol (0·5 ml) was added a solution of 1,3,5-trinitrobenzene (66 mg) in ethanol (5 ml). A trinitrobenzene adduct, vermilion needles, m.p. 136–137° (recrystallized from ethanol three times), which was identical with a trinitrobenzene adduct of XV,[4d] was obtained. The spectra of XV, ν_{max}^{film} 3473, near 3400 (broad) and 1218 cm^{-1} (for phenol); λ_{max}^{alc} 235·5 (ε 37·950), 305·5 (ε 6275), 320 (ε 5520) and 337 mμ (ε 5780) (see Fig. 2).

10-*Acetoxy*-2,5-*dimethyl*-8-*isopropyldecalone*-1 (XIII)

The preferred method of acetylation was with isopropenyl acetate: a mixture of this reagent (1 ml), *p*-toluenesulphonic acid monohydrate (1·2 mg) and ketol (XI) (102 mg) was heated (steam bath) for 4 hr with protection from moisture. The mixture was neutralized with a solution of a few pieces of sodium acetate in water (3 ml), evaporated *in vacuo*, extracted with ether, washed with water, saturated potassium bicarbonate solution and water, dried (Na_2SO_4) and evaporated giving colourless needles (116 mg). Recrystallization from petroleum ether gave XIII as colourless needles, m.p. 130–131°, $[\alpha]_D^{18}$ +85·5° (±2°) (c 1·00, ethanol), ν_{max}^{Nujol} 1724, 1703, 1260, 1238 and 1017 $cm.^{-1}$ (Found: C, 73·02; H, 10·03. $C_{17}H_{28}O_3$ requires: C, 72·82; H, 10·06%).

2,5-*Dimethyl*-8-*isopropul*-Δ^{9-10}-*decalone*-1 (XII)

(1) *By dehydration of* XI. To a solution of Na (460 mg) in ethanol (10 ml) was added XI (104 mg). The solution was refluxed in nitrogen atmosphere for 4 hr, then evaporated *in vacuo*, extracted with ether, washed with water, dried (Na_2SO_4) and evaporated leaving a pale yellow oil (88·1 mg). The residue was distilled at 115–116°/2 mm yielding a colourless oil, $[\alpha]_D^{21}$ −30·5° (±2°) (c 1·898, dioxane), λ_{max}^{alc} 247·5 mμ (ε 11200), ν_{max}^{film} 1665 and 1622 cm^{-1}. (Found: C, 81·46; H, 11·01. $C_{15}H_{24}O$ requires: C, 81·76; H, 10·98%).

(2) *By pyrolysis of acetoxy-ketone* (XIII). The apparatus consisted of a quartz-tube, 200 mm high with a 10 mm i.d., packed with Pyrex ball, 2–3 mm dia. was used. XIII (500 mg) was added at the rate of 15–20 mg/min to the top of the pyrolysis tube heated at 400–450°, during which time the addition was conducted in an inert atmosphere by introducing a slow stream of nitrogen at the top of the tube. The pyrolysate was dissolved in ether, washed with water, saturated potassium bicarbonate and water, dried (Na_2SO_4) and evaporated leaving a colourless oil (420 mg). The residue was crystallized with the addition of petroleum ether giving the starting material (90 mg). The mother liquid was distilled at 114–116°/2 mm yielding a colourless oil (160 mg), which was shown to be identical with XII by infrared comparison, but showed $[\alpha]_D^{21}$ −9·2° (±3°) (c 0·837, dioxane), λ_{max}^{alc} 247·5 mμ (ε 9170).

Ozonolysis of the VI*-rich fraction*

A solution of VI (1·48 g, the ratio of the peak heights at 8·8 and 11·25 min was 1 : 2·97) in ethyl acetate (20 ml) was ozonized at −75° in the usual manner. After removal of the solvent under diminished pressure at room temp, zinc dust (1 g) was added in small portions to a solution of the ozonide in glacial acetic acid (8 ml) with stirring (ice bath), and then the mixture was filtered, evaporated *in vacuo*, extracted with ether, washed with water, 2 N NaOH and water, dried (Na_2SO_4) and evaporated giving a pale yellow oil (1·22 g). This oil was distilled at 116–120°/1·5 mm giving XVI (609 mg), ν_{max}^{film} 1709, 1410, 1388 and 1369 cm^{-1} which showed negative results to Fehling's and Tollen's reagents and positive result to iodoform-test for CH_3CO-group.

Treatment of XVI *with* 0·1 N *sodium methoxide in methanol*

To a solution of Na (23 mg) in methanol (10 ml) was added XVI (287 mg). The mixture was refluxed in nitrogen atmosphere for 2 hr giving a yellow oil (203 mg), which was distilled at 96–97°/0·5 mm yielding XVII (172 mg), λ_{max}^{alc} 244·5 mμ (ε 10850).

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TERPENOIDS—XXV*

STRUCTURE OF SAUSSUREA LACTONE

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Abstract—Saussurea lactone is identical with the product of pyrolysis of dihydrocostunolide. On cyclisation in the presence of p-nitrobenzoic acid followed by hydrogenation it gives santanolide C. Hence structure (V) is assigned to saussurea lactone.

COSTUS root oil obtained from the plant *Saussurea lappa* C. B. Clarke has been well known for its lactonic constituents. Of these, dehydrocostus lactone,[1] costunolide (I)[2,3] and 12-methoxy-dihydrocostunolide(II)[4] have been completely characterized. From vacuum distilled (60–210°/11 mm) costus root oil Rao and Varma[5] isolated a crystalline lactone which they have named as saussurea lactone. In a subsequent communication Rao *et al.*[6] suggested a tentative bicyclic structure (III) for this lactone on the basis of degradative studies. The possibility of the santenolide type of structure (IV) was not entirely ruled out. According to them, the lactone has the molecular formula $C_{15}H_{22}O_2$ and contains one double bond of the type $=CH_2$. They obtained 1-methyl-7-ethyl naphthalene on selenium dehydrogenation.

Investigations described in the present communication show that saussurea lactone is to be represented by the structure (V).

At the initial stages of our investigations, it was felt that a comparison of fully hydrogenated product from saussurea lactone (V) with the various santanolides or alantolides would be of help in firmly establishing its structure and possibly in elucidating its stereochemistry, but this did not lead to any fruitful result, as fully hydrogenated saussurea lactone could not be identified with any of the santanolides or alantolides. It was therefore felt that the structure of saussurea lactone required a more critical examination.

Earlier authors[6] have described saussurea lactone as a monoethynoid compound, but more careful examination showed that saussurea lactone does not contain one double bond, but contains two double bonds and hence is a monocarbocyclic compound and not a bicyclic product as previously postulated. Consequently the previous structure (III) required revision.

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† Indian Standards Institution, Delhi.

[1] M. Romanuk, V. Herout and F. Sorm, *Coll. Czech. Chem. Comm.* **21**, 894 (1956) and references cited therein.
[2a] A. Somasekar Rao, G. R. Kelkar and S. C. Bhattacharyya, *Tetrahedron* **9**, 275 (1960); [b] *Chem. & Ind.* 1359 (1958); [c] *Chem & Ind.* 1069 (1959).
[3] V. Herout and F. Sorm, *Chem. & Ind.* 1067 (1959).
[4] Part XXIV: *Tetrahedron* **12**, 178 (1961).
[5] P.S. Rao and B. S. Varma, *J. Sci. Ind. Res.* **10B**, 166 (1951).
[6] P. S. Rao, B. S. Varma, N. R. Ghosh and P. C. Dutta, *J. Sci. Ind. Res.* **17B**, 228 (1958).

The I.R. spectrum of saussurea lactone (V) showed the presence of both types of methylenic double bonds (i) R.CH═CH_2 (909 cm^{-1}), (ii) R—C(R′)═CH_2 (892 cm^{-1}). In view of this and the conversion of saussurea lactone (V) to 1-methyl-7-ethyl naphthalene it was suspected that saussurea lactone may have the elemane type of carbon

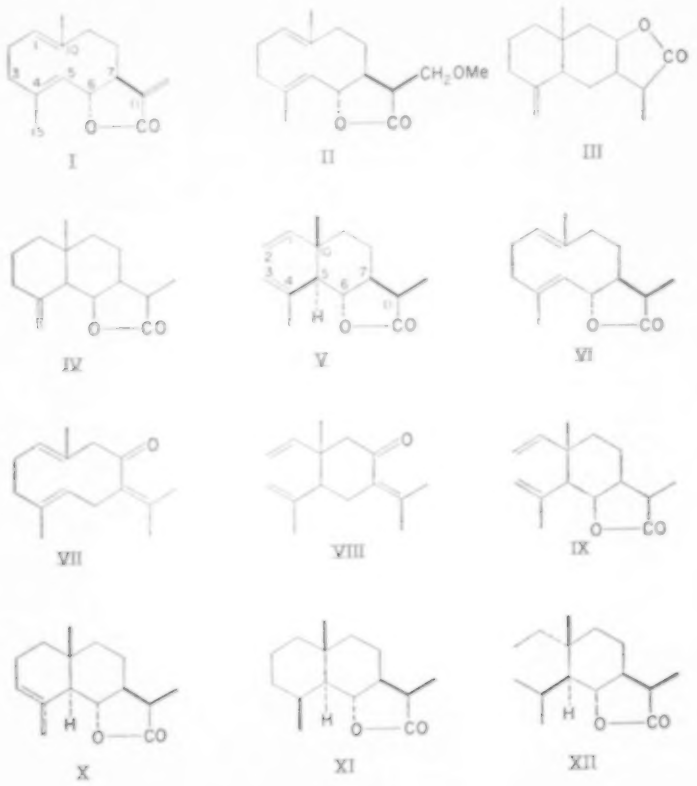

FIG. 1.

skeleton. Rao and Varma[5] obtained saussurea lactone (V) from costus root oil distilled at high temperature. In connexion with our work,[7] involving large amounts of costus root oil isolated and processed under very mild conditions without employment of any thermal treatment and involving mostly column chromatography, we failed to isolate any saussurea lactone, but such oil contains large amounts of costunolide (I) and also appears to contain dihydrocostunolide (VI). Hence it was felt that saussurea lactone (V) was derived from the pyrolysis of the latter (VI).

To prove the validity of this assumption pyrolysis of dihydrocostunolide (VI) prepared from costunolide (I) was carried out and the product was found to be identical with saussurea lactone (V). On the basis of the earlier work dealing with the

[7] A. Paul, A. S. Bawdekar, R. S. Joshi, G. H. Kulkarni, A. Somasekar Rao, G. R. Kelkar and S. C. Bhattacharyya, *Perf. & Ess. Oil Rec.* **51**, 115 (1960).

pyrolysis of germacrone (VII) to pyrogermacrone (VIII)[8,9] the gross structure (IX) can be assigned to saussurea lactone. Such a structure is also consistent with its I.R. spectrum discussed earlier. In addition, this also establishes the absolute configuration of saussurea lactone at C_6, C_7 and C_{11} since the absolute configuration of dihydrocostunolide (VI) is known.[2,3]

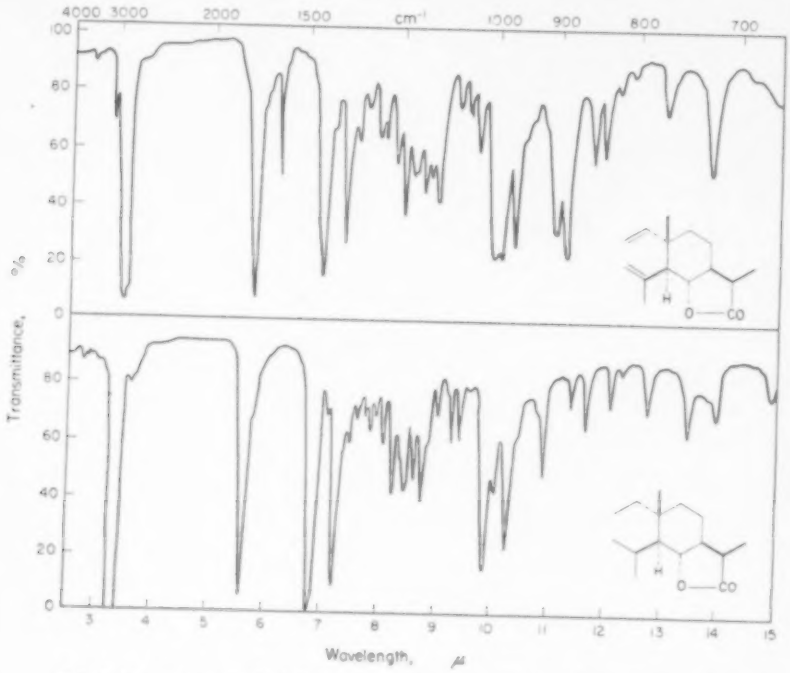

FIG. 2. I.R. spectra (in Nujol) of saussurea lactone and tetrahydrosaussurea lactone.

To determine the absolute configuration at the remaining asymmetric centres, C_{10} and C_5 saussurea lactone (V) was cyclized by heating with *p*-nitrobenzoic acid[10] to a monoethynoid bicyclic product. The exact position of the double bond in the cyclized product (X) has not been determined; but the U.V. and the I.R. spectra of the cyclized product excluded the presence of the Δ^4-isomer (tetrasubstituted double bond) and indicated that it was a mixture of Δ^3 and $\Delta^{4(15)}$ isomers in which the former predominated. This is supported by a comparison of the m.p. and rotation of the cyclized product with those of the Δ^3 isomer reported previously by Cocker and McMurry[11]. It became necessary to investigate this aspect as an unsaturated lactone with the double bond between carbon atoms C_4 and C_5 cannot be of much value in determining the absolute configuration at C_5. The cyclized product (X) on hydrogenation gave santanolide C.[2,3] This leads to the unambiguous assignment of the absolute configuration to saussurea lactone (V) at all the centres of asymmetry.

[8] I. Ognjanov, V. Herout, A. Horak and F. Sorm, *Coll. Czech. Chem. Comm.* **24**, 2371 (1959).
[9] G. Ohloff, H. Farnow, W. Phillipp and G. Schade, *Liebigs Ann.* **625**, 206 (1959).
[10] W. Wicki, J. Kalvoda and O. Jeger, *Croatica Chem. Acta* **29**, 263 (1957); *Chem. Abstr.* **53**, 16197e (1959).
[11] W. Cocker, and T. B. H. McMurry, *J. Chem. Soc.* 4549 (1956).

It would appear that saussurea lactone (V) is not a natural constituent of costus root oil, but is an artefact formed by pyrolysis of dihydrocostunolide (VI).

EXPERIMENTAL

All melting points are uncorrected. Rotations were taken in chloroform. Ultra-violet spectra were determined in alcohol solution on Beckman DK-2 recording spectrophotometer. Infra-red spectra were determined with Grubb Parson spectrophotometer and Perkin–Elmer infracord spectrophotometer. Microanalyses were carried out by Mr. Pansare and colleagues.

Saussurea lactone (V)

The saussurea lactone used at the initial stages of the present work was obtained from the personal collection of samples of Dr. Sadgopal. It was crystallized repeatedly from methanol. m.p. 148–149°, $[\alpha]_D = +66°$, lit.[5,6] records for saussurea lactone m.p. 146–147°, $[\alpha]_D$ +57°, +62°. (Found: C, 76·9; H, 9·6. $C_{15}H_{22}O_2$ requires C, 76·88; H, 9·46%). U.V. spectrum: no peak in the region 210–340 mμ, ε_{210}, 1300.

I.R. spectrum (in Nujol): Bands at 1760, 1633, 1450, 1374, 1334, 1310, 1276, 1258, 1228, 1207, 1187, 1178, 1158, 1146, 1127, 1080, 1060, 1041, 1007, 996, 974, 943, 909, 892, 856, 840, 826, 812, 774 and 727 $cm.^{-1}$

Hydrogenation of saussurea lactone (V)

Saussurea lactone (0·814 g) was hydrogenated in acetic acid solution in the presence of platinum oxide (80 mg) catalyst. The amount of hydrogen taken up (180 ml at 27° and 710 mm) corresponded to the presence of two double bonds. The catalyst was filtered off. From the filtrate acetic acid was removed on steam bath with water pump. The residue was taken up in ether and washed with sodium carbonate solution. The solid left after removal of ether was crystallized three times from ethanol and sublimed to give the tetrahydrosaussurea lactone (XII), m.p. 123–125°, $[\alpha]_D^{27} = +42°$ (C, 7·0) (Found: C, 75·8; 75·8; H, 10·9; 11·2. $C_{15}H_{26}O_2$ requires C, 75·58; H, 11·00%).

I.R. spectrum (in Nujol): Bands at 1760, 1444, 1374, 1333, 1315, 1285, 1271, 1257, 1236, 1207, 1175, 1158, 1139, 1124, 1102, 1073, 1057, 1037, 1005, 991, 968, 914, 873, 853, 823, 811, 782, 742, 714 cm^{-1}.

Pyrolysis of dihydrocostunolide (VI)

Dihydrocostunolide (VI) was prepared from costunolide by following the procedure described earlier.[24] It had m.p. 77–78°, $[\alpha]_D$ + 114°.

Dihydrocostunolide (1 g) was taken in a sublimation apparatus and heated at 200–220° at 50 mm for 20 min. Subsequently the pressure was reduced and the sublimation completed. The process was repeated with the sublimed product once more. The material obtained after two sublimations was crystallized three times from ethanol and again sublimed (120°/0·5 mm) to give a solid (yield 0·4 g) m.p. 147–148°, mixed m.p. with saussurea lactone was undepressed. $[\alpha]_D = +66°$ (C, 3·0). (Found: C, 77·3; H, 9·7. $C_{15}H_{22}O_2$ requires C, 76·88; H, 9·46%). I.R. spectrum was identical with that of saussurea lactone.

Cyclization of saussurea lactone (V)

A mixture of saussurea lactone (0·75 g) and *p*-nitrobenzoic acid (0·5 g) was heated at 180–190° for 4 hr in nitrogen atmosphere. The reaction product was taken up in ether and washed with sodium carbonate solution to remove *p*-nitrobenzoic acid. Ether was evaporated from the neutral ether extract and the residue was sublimed (120°/0·5 mm). It was then crystallized three times from ethanol and again sublimed to give the cyclized product (X) (yield 0·31 g); m.p. 129–131°, $[\alpha]_D = +109°$ (C, 3·3), lit.[31] records for sant-3-enolide m.p. 136–137°, $[\alpha]_D = +91°$. (Found: C, 76·72; H, 9·45. $C_{15}H_{22}O_2$ requires C, 76·88; H, 9·46%); U.V. spectrum ε_{210} 2200; ε_{215} 1000; ε_{220}, 350.

Hydrogenation of the lactone (X)

The cyclized product (X) (0·20 g) from the above experiment was hydrogenated in acetic acid in the presence of platinum oxide (50 mg) catalyst at room temperature and atmospheric pressure and hydrogen approximately corresponding to one double bond was absorbed. The hydrogenation product was worked up in the usual way. It was crystallized twice from ethanol and sublimed to give 130 mg of a solid which was identified as santanolide C from its melting point and mixed m.p. with an authentic sample 151–153°, $[\alpha]_D = +56°$ and identity of its I.R. spectrum with that of santanolide C. (Found: C, 75·9; H, 10·0. $C_{15}H_{24}O_2$ requires C, 76·22; H, 10·24%), lit.[8a,3] records for santanolide C, m.p. 154–155°, 153–154°, $[\alpha]_D + 55°$.

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Tetrahedron, 1961, Vol. 13, p. 324. Pergamon Press, Inc. Printed in Northern Ireland

BOOK REVIEWS

LARS MELANDER: **Isotope Effects on Reaction Rates.** Ronald Press, New York, 1960. 181 pp. $6.00.

THIS small book is described by the author as "a brief presentation of the main principles of kinetic isotope effects, with no attempt being made to cover the entire field". It is the best review now available, and most workers in this field will want a personal copy for convenient reference.

Chapter 2 on general theory is excellent. It assumes a knowledge of the material on transition state theory covered by Frost and Pearson in *Kinetics and Mechanism*, 1953, Chap. 5, but proceeds clearly from that point. There are astonishingly few errors in the book. The only one detected in this chapter was a relation between imaginary frequency and curvature of the potential energy surface (rather than square root of this curvature) on p. 11.

Chapter 3 on mathematical methods for evaluation of isotope effects from experimental data is hard reading, but especially valuable for reference, serving a similar function in this book to Chap. 8 in Frost and Pearson on analysis of complex non-isotopic reactions.

Chapters 4–6 on hydrogen isotope effects are less valuable in view of the excellent and more detailed earlier review by Wiberg [*Chem. Rev.* **55**, 713 (1955)], but do include more recent references.

Chapters 7–9 on heavy atom (C, N and S) isotope effects are written primarily from the approach of a physical chemist rather than of an organic chemist concerned with establishing reaction mechanisms. This reviewer was surprised to find no references to the work of Bourns, Fry or Saunders. These are the people whom he would have thought of first as the pioneers in the area of heavy atom isotope effects for mechanistic studies. Nevertheless there are 106 references, including 24 in 1958 and 4 in 1959, and there is a good five-page index.

C. GARDNER SWAIN

Progress in Drug Research. Edited by ERNST JUCKER (Editor): Virkhauser Verlag, Basel and Stuttgart, 1959. 607 pp., 68 Sw. Fr.

THIS first volume of a new series *Progress in Drug Research* was in fact published in 1959 but has just been received by the reviewer. It is planned to bring out a new volume annually and each is to contain some six to eight chapters written by authorities in the particular fields. As the science of drugs covers a very extensive field, the articles may extend to pure chemistry through pharmacology to medicine and in fact such a spread is evident in this volume. It follows therefore that the book is uneven and the mode of treatment of the various topics varies widely although each endeavours to give a certain amount of background so that the reviews are not designed only for specialists. The first chapter is an extensive treatment (115 pp.) of ion exchange resins and their use in pharmacy and medicine; this review like all but two in the book is written in the German language, the others being in English. There follows a shorter account of cholesterol and its relation to atherosclerosis and an extensive treatment of the biology and chemotherapy of worm infections. An account of the various drugs used as anthelmintics is followed by the longest chapter (177 pp.) in the book dealing with the placebo problem. The last two chapters stand in sharp contrast. A. H. BECKETT gives an interesting account of the importance of stereochemistry, the shape of the molecules, in the design and biological activity of drugs and attempts to give a more logical approach to the design of new substances of chemotherapeutic value. The final chapter is a non-critical catalogue of the most important drugs discovered in the last five years. All chapters are fully documented with up-to-date references (numbered separately on each page) to the original literature and it is clear that the editor has succeeded in providing a useful and valuable cross-section of the very wide literature of this important science. No author or subject index is provided. The general format and presentation of the book are excellent.

A. W. JOHNSON

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